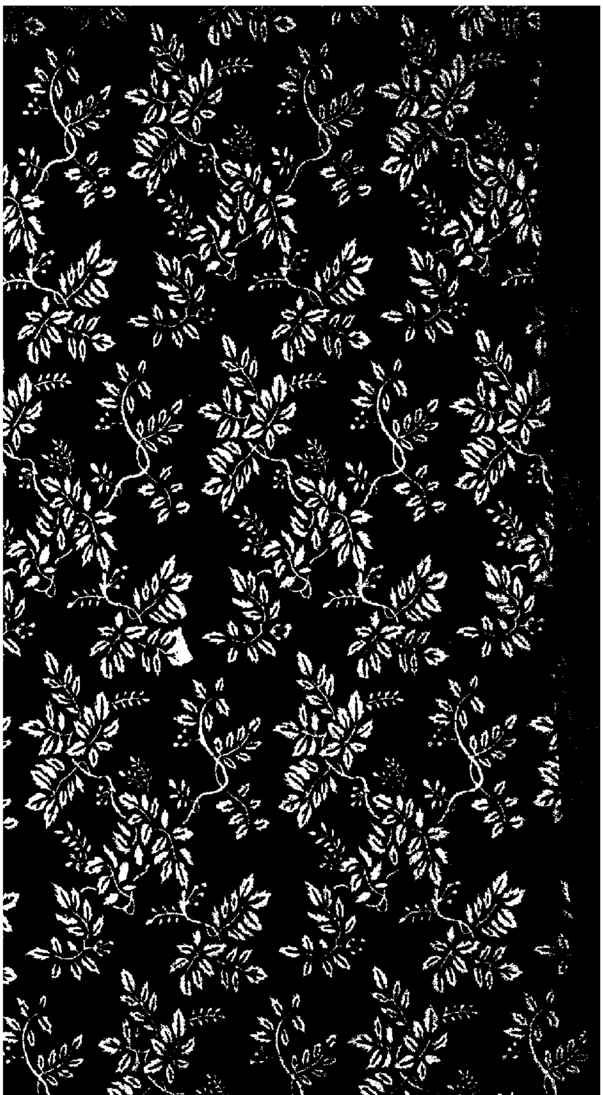

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Contents

Part 3 (December, 1907).

Part 4 (July, 1908).

CONTENTS.

Part 1 (January, 1907).

Part 2 (April, 1907).

THE IMPROVEMENT OF ENGLISH WHEAT.

A RÉSUMÉ BY

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THE following *résumé* of the work so far published by the Home-grown Wheat Committee of the National Association of British and Irish Millers is based upon a paper presented by one of us to the International Convention of Millers which met in Paris during October, 1905[1]. This work being well past the tentative and preliminary stages it proved possible to make a broad survey of it and to piece together isolated special portions which have been published from time to time.

Its publication has two objects in view: one to bring the work to the notice of agriculturists to whom otherwise it might be inaccessible, and the other to persuade those who recognize what results may accrue from a many-sided investigation of this kind to take up the study of our other farm crops on somewhat similar lines. Many will reply that such work may well be left in the hands of the professional seedsmen, but if they will look back upon the history of any one of our farm crops and consider the improvements, or otherwise, made during the last twenty years they will probably arrive at a different conclusion. The story of wheat, *mutatis mutandis*, is much the same as that of many of our crops, and where as in this country large monetary returns per acre are essential the quality of the crops can no longer be neglected.

[1] "The Improvement of English Wheat," by A. E. Humphries, published by the Incorporated National Association of British and Irish Millers, 59, Mark Lane, London, E.C.

Plant-improvement has in the immediate past been too much associated with efforts to obtain greater yield, shapeliness, colour, etc., whilst results of great value are to be won by seeking for that most subtle of attributes, quality.

As far back as the year 1890 the National Association of British and Irish Millers called attention to the fact that the quality of our home-grown wheats had deteriorated, and pointed out that "such wheats bring but a poor price and require a large admixture of strong foreign wheats to make a flour that will bake into a satisfactory loaf of bread." At this period the older varieties, such as Chidham, Rough Chaff, and Lammas, were being supplanted by varieties of the Square Head and Stand-Up types, whilst the growing importation of strong wheats had set up a much higher standard of quality. The appeal circulated by the Association had little or no effect, and as a result of the deterioration of quality and the simultaneous steady decline in the acreage devoted to wheat many of the inland mills went out of existence. In the following years numerous new varieties of wheat were introduced, many of them claiming to possess excellent milling qualities, yet in 1900 the Association found it necessary to again call attention to the fact that the quality of our English wheats was still deteriorating. They attributed this to the fact "that farmers and seed-raisers pay more attention to the large quantity of straw and wheat produced by some of the newer varieties, whilst overlooking the fact that many of these are singularly destitute of gluten and of other characteristics which are of the utmost value for milling purposes." To anyone inspecting the wheats at our agricultural shows it will be clear that this is still the case to-day. The large berry, which is soft and obviously full of starch, is still the judge's ideal.

Efforts were also made by the Millers' Association to induce the most prominent agricultural societies throughout the kingdom to take the matter up, but, though it was all too clear that the English varieties then grown could not compete with the foreign grain coming in ever-increasing quantities into the country, and that consequently the prices obtained by the home producer were and must continue to be unsatisfactory, nothing was done.

This state of affairs was not confined to Britain, but prevailed in other countries in Western Europe. In Denmark, for instance, our square-headed wheats were introduced about 1880 with the result that this same complaint of lack of strength was quickly made. A committee appointed by the Royal Agricultural Society of Denmark began

to investigate the question in 1882 and reported in 1896 that it was not profitable under their conditions to grow wheats of greater strength and pay for it with a smaller yield. If strength and low yield are correlated then it may be that all attempts to obtain strong wheats suitable for our conditions are doomed to failure, but of this assumption there is no proof whatever.

In 1901 the National Association of British and Irish Millers despairing of help from the agricultural societies of the country called into existence a small committee representing both the milling and agricultural industries, supplying it to the best of its ability with funds to investigate the possibilities of improving our home-grown wheats. Since the inception of the work it is pleasant to note that many of the experimental stations both at home and abroad have willingly given their assistance, and that the Board of Agriculture have from time to time provided the committee with additional funds. The ultimate object of the committee's work has been to produce strong varieties of wheat of good yielding power suitable for our English conditions of cultivation, since the varieties now in existence, excellent as they are in many respects, are without exception lacking in the strength which modern standards of bread-making demand.

We must at the outset define "strength." The term has been used in various ways by different authorities with the result that the study of the literature dealing with such problems has become somewhat confused. Many writers, particularly on the Continent, estimate strength by the quantity of water a given quantity of flour will absorb in producing dough of a standard consistency, the consistency being measured by various methods, such as its stretching power, its resistance to puncturing, etc. In practice such methods are unsatisfactory, for the bakers do not make the various kinds of flour up to one and the same consistency in the doughs. To give the best possible loaves some require to be made into "tight," others into "slack" doughs, and the baker simply learns by experience what particular degree of consistency is the most suitable for the flour he has in hand. Another common method of attempting to give some idea of the strength of flour is to associate it with the number of loaves a given quantity of flour (in practice a sack) will produce. The larger the number of the loaves produced the stronger the flour is considered to be. Under such circumstances the suitability of the loaf from the consumer's standpoint, if not from the baker's, is neglected. Some Russian and most Indian wheats, for instance, give a large numerical

yield per sack, but the loaves are small and close in texture. A third view, apparently largely adopted by the bakers, is to judge strength by the way a flour behaves in the doughs, by its toughness, elasticity, freedom from stickiness, etc.; in other words, by the facility with which large masses of dough can be handled in the bakehouse. It seems more satisfactory to regard these as separate characteristics, for though of undoubted importance to the baker they are not necessarily associated with the production of satisfactory loaves. The fact that some of the Russian wheats from St Petersburg or Reval are esteemed strong, but work very badly in the doughs, will show the necessity for this distinction.

The definition finally adopted by the committee is, that a strong wheat is one which yields flour capable of making large, well-piled loaves, the latter qualification thus excludes those wheats producing large loaves which do not rise satisfactorily. To estimate the strength of any particular sample of wheat then it is necessary to grind it and make the final tests in the bakehouse.

In order to minimize as far as possible the chances of errors involved in baking tests certain precautions have been observed throughout. In the first place the baking trials are made with sufficient flour to yield a batch of about half-a-dozen loaves—the "cottage" shape being considered the most satisfactory. With each set to be tried loaves are baked from flour whose quality has been accurately ascertained. To these standard loaves a certain number of marks are assigned, and by comparison the baker records in marks his opinion of the strength of the flour under test. On this arbitrary scale the strongest wheats in commerce mark about 100, "London Households" 80 to 85, and average English 60 to 65. The tests are always carried out by a man who devotes the whole of his time to this kind of work, and repeated trials have shown that they may be relied upon to express the strength with substantial accuracy.

It was foreseen, however, that this process would be impracticable on a very large scale, if only on account of the labour involved in growing sufficiently large plots of the many varieties of wheat the committee anticipated they would have to deal with. Other methods for estimating strength were therefore sought for.

From time to time various methods have been suggested for this purpose. These have been subjected to a critical examination at Rothamsted[1]. One of the oldest methods assumes that the percentage

[1] Hall, *Journal of the Board of Agriculture*, Vol. XI. p. 321.

of gluten extracted from a flour by washing is an index of its strength. In certain cases it was found that the strength determined by the gluten test and by baking trials agreed, but so many exceptions occurred that it was evident that the method could not be relied upon. It appears to be rather the quality of the gluten than its amount which determines strength. The character of the gluten itself has been invested by Girard and Fleurent, and they have come to the conclusion that the relative proportions of gliadin and glutenin contained in it determine its quality. In their opinion a ratio of 75 of gliadin to 25 of glutenin is the criterion of a good flour.

Guthrie in New South Wales and Snyder in Minnesota also make use of the gliadin-glutenin ratio as a test for strength. The latter considers the ratio of 65 : 35 indicates a well-balanced gluten. The difference between these figures of Fleurent and Snyder is mainly due to differences in the method of estimating gliadin. The value of this test has been open to criticism from the first. On comparing the results obtained by the gliadin-glutenin ratio with those of the bakehouse there appears to be "no agreement whatever between them, when flours of widely differing characteristics are being tested."

On the whole the estimation of the total nitrogen percentage in either the grain or the flour gives results which are nearest to agreeing with the baking trials, those wheats with the highest nitrogen content being the strongest. A certain amount of caution has to be used in applying the test, for it is by no means an absolute measure of strength. For instance, the highest nitrogen content in a series of trials with Square-Head's Master grown on different soils was 1·97, and its baking marks 40, whilst a better sample marked at 62 had a lower nitrogen percentage, namely 1·66. Where the wheats are grown under similar conditions the nitrogen content appears to be a satisfactory index of their strength.

One of the first problems attacked was that of the cause of strength in wheat. It is popularly supposed in this country and in many parts of Western Europe that wheats are poor in quality owing to climatic conditions. Here, for instance, it is often stated that lack of sunshine and the poverty of the soil compared with the virgin lands of the wheat-growing districts of Canada and the United States are the controlling factors in making our wheat so deficient in strength. If this were to prove the case then the possibilities of improving the quality of English wheat would be slight, but many facts are known which militate against this view. The fact that India, Australia, and California export wheats

no stronger than our own makes it clear that abundance of sunshine does not necessarily result in the production of strong wheat, and a comparison of the strength of wheat grown in the wet and sunless season of 1903 with that of the sunny season of 1904 showed on the whole a slight difference in strength there in favour of the year with less sunshine. In short it may be affirmed that climatic conditions, such as amount of sunshine, temperature at various stages of growth, rainfall, or the amount of moisture present in the air have no such noteworthy effect on strength that we need despair of growing strong wheats in this country. Investigations on the influence of soil on strength have been carried out in the following manner. Two varieties of wheat, Red Lammas and Square-Head's Master, were grown side by side on seven typical soils. Red Lammas is one of our best varieties, whilst Square-Head's Master is of indifferent quality. The plots were harvested as nearly as possible under the same conditions of ripeness and a portion of the wheat ground and tested in the bakehouse. In the following table the results are expressed by the baker's marks:

Soil	Lammas	Square-Head's Master
Warp	70	62
Deep loam	65	40
Fen	65	55
Sand	62	55
Deep sandy loam	58	42
Thin chalky loam......	57	30
Stony clay	50	40
Average.........	61	46

from which it is evident that although the better samples of Square-Head's Master were superior to the worst samples of Lammas yet on every soil the Lammas proved itself the stronger variety of the two. The table further shows that soil conditions have a considerable influence on the strength of wheat, though it gives no information as to the precise factor or groups of factors determining it. Neither does it follow from these results that warp soil will invariably grow good, and thin, chalky soils poor quality wheat, for in the case of another variety, Fife, this latter soil has produced wheat of really great strength. It will therefore be necessary to find varieties capable of giving the maxi-

mum of strength under strictly local conditions, and this problem each locality may have to solve for itself.

Once it was proved that the soil in some way influences strength, the question arose as to whether it would be possible to secure improvements in this direction by manuring. The wheat plots at Rothamsted and Woburn offered unique facilities for such an investigation, and with the cooperation of the trustees of the Rothamsted Experimental Station and of the Royal Agricultural Society of England, this was commenced. The results obtained up to the present are surprising in many ways. A brief summary of the more important ones are given in the following table:

Rothamsted			1903 crop			1904 crop	
Broadbalk Field Plot No.	Dressings	Yield per acre	Strength Nov. 17, 1903	Strength May 12, 1904	Strength June 28, 1904	Strength Autumn, 1904	Strength Spring, 1905
3	Unmanured	12·8	50	50	55	45	60
10	Amm. Salts = 86 lbs. N.	20·4	3	30	48	20	38
11	,, + Super	23·6	0	30	45	20	45
7	,, ,, + Minerals	32·6	below 0	below 0	—	10	35
2b	Farmyard Manure	35·3	below 0	below 0	below 0	—	—

In both the years 1903 and 1904 the wheat from the unmanured Rothamsted plots was considerably superior in strength to that of the manured plots. Thus after the wet harvest of 1903 in the bakehouse the unmanured plot earned 50 marks, plot 10 with ammonium salts 3, plot 11 with ammonium salts and superphosphate 0, plot 7 with a "complete dressing" below zero, and 2*b* with farmyard manure below zero. In 1904 the corresponding marks for these plots were 45, 20, 20, 10 and below zero. Strangely enough the wheat from the highly manured plots actually appeared to the eye to be stronger than that from the unmanured, and analysis showed that the use of nitrogenous manures had increased the percentage of nitrogen in the grain. Further baking trials brought out the fact that the strength of the grain from the highly manured plots was increasing abnormally with its age. On Nov. 17th, 1903, the unmanured plot earned 50 marks and 55 on June 28th, 1904, or practically its quality was unaltered, whilst plot 10, marked 3 on the former date, had risen gradually to 48 on the latter, when further improvement ceased. The 1904 crop showed the same

result though the differences were not so obvious. This marked improvement in quality on ageing coincided with a deficiency of phosphates in the ash of the grain.

The results obtained on the light soils of Woburn differ radically from those at Rothamsted, although the strength of the wheat on the unmanured plot was practically the same at both stations. Without describing them plot by plot it may simply be stated that the strength was practically unaffected by any form of manuring, and that there was no noteworthy development of it after ageing. The differences in the results are difficult to explain, but the experiments show satisfactorily that long-continued heavy manuring does not increase the strength of wheat, and it may even seriously depreciate it.

Many of the districts producing strong wheat have, owing to the severity of their winters, to resort to the cultivation of rapidly growing types which will mature satisfactorily when spring-sown. This is the case for instance with the wheats of Minnesota, Dakota, Manitoba, and also with Ghirka wheat. This fact may possibly explain the view so generally held in this country that spring-sown wheats are stronger than autumn-sown ones. But that spring-sowing can be an actual source of strength appears to be negatived by the fact that some Russian wheats, such as Azima, are autumn-sown, and that the same is true of Kansas and some of the Hungarian wheats. This question of a possible correlation of strength and rapid maturation has, however, been investigated in some detail, in the first place in the hope of obtaining some information on the factors regulating the production of strong grain, and further because a really satisfactory spring wheat, if found, would be a useful standby to farmers when unsuitable conditions prevailed for autumn planting.

Most of the trials had to be made with foreign wheats owing to the fact that so few of our varieties mature satisfactorily when spring-sown. This proved to be the case with Scholey's Square-Head for instance.

All of the evidence obtained so far tends to show that no tangible increase of strength results from spring-sowing. For example in 1903 ten different sorts of wheat were spring- and autumn-sown on various experimental farms. As some of these sorts were grown at two or more places fifteen distinct pairs of baking trials were possible. In no case did the spring-sown wheat prove appreciably stronger than the autumn-sown, the baking marks being practically level in each case. The average mark for the series was 83 for the autumn-sown and 83 for the spring-sown. A second series of eight sorts grown by one of us

in 1904 gave the same result, the spring- and autumn-sown wheats earning 77 and 79 marks respectively. As 1903 and 1904 were typically wet and dry seasons the results may be taken to hold for a wide range of climatic conditions. A further trial made with Nursery wheat which is one of the few English varieties suitable for the purpose was conducted on slightly different lines. The grain was sown in the middle of November, and again at monthly intervals until April. With the exception of the April-sown plot, which had to be abandoned, a satisfactory plant was obtained in all cases. On testing the five remaining plots the strength was found to be practically the same in all.

In addition to the baking results of these various trials one of us has recorded the number of days from sowing to earing, earing to forming grain, forming grain to ripening, together with the average daily temperatures throughout this period. The mass of statistics, for which the original paper[1] must be consulted, show that under our conditions the importance of rapid maturation has been much overestimated. In 1903 for instance the strongest of the autumn-sown wheats averaged 58 days from earing to ripening, whilst some of the weakest took the same, or even a less, time. A few figures taken from the 1904 trials will show further how little connexion there is between strength and rapid maturation. Three strong wheats marking 92, 87, and 84 respectively matured in 42, 45, and 44 days, and three weak ones marking 67, 63, and 61 in 42, 42, and 40 days. The shortest period between earing and ripening was 38 days, and this particular variety only earned 65 marks in the bakehouse. The weakest wheat in the series marked as 45 took 48 days to mature.

The strength of wheat has for many years been considered to be largely dependent on the time at which it was harvested. If this were the case and no marked depreciation in yield resulted from an early harvest it is clear that it would be advantageous to investigate the most suitable stages at which the crop should be cut. The first case examined was that of Rivet wheat cut when dead ripe, normally ripe, and green. The two latter samples were grown on the same farm, but the first on a different one. On baking, the three samples were marked 15, 35, and 52 respectively. The total nitrogen percentage and the gluten tests also indicated that the material cut green had about the average strength of English wheat, whilst the corresponding figures at the dead-ripe stage pointed to its being greatly deficient in strength. An independent trial gave the same results, the expert reporting that

[1] Humphries, "Improvement of English Wheat."

the dead-ripe sample baked as if the gluten had previously been washed from it. Although this affords strong confirmation of the popular belief in the value of early cutting it was considered advisable to make further trials, so, in the following seasons, samples of different varieties of wheat were obtained from various localities, each set, representing the three stages, being cut from the same piece of wheat. For this trial two sets of Square-Head's Master from different localities, Stand-Up White, and Red Giant were milled. In each case the baking marks for each particular variety showed that there was practically no difference in the strength of the grain at these three stages. There appears to be no reason why the results with the Rivet series should be mistrusted, but as it is desirable to confirm them, experiments are now in hand for this purpose. It should be noted that Rivet wheat belongs to a different sub-species of wheat from those commonly cultivated in this country, and it may be that these marked differences in its strength at various stages of ripening are peculiar to it[1].

From the foregoing account it is clear that although strength can be influenced to a certain extent by the conditions under which wheat is cultivated, the greatest improvements to be obtained by varying these conditions are not likely to be sufficient to bring the strength of even our best varieties to the level of that of such wheats as the Manitoban. It is therefore of great interest to know to what extent the strong foreign wheats can profitably be cultivated in this country, and whether any could be found suitable for our purposes. The work which had already been carried out on the Continent, more particularly that of the Danish Royal Agricultural Society, tended to show that the chief objection to cultivating the strong wheats of other countries was their rapid deterioration under the new conditions, and it did not hold out much promise for the success of such experiments here. Nevertheless the investigation was undertaken by the Committee and is still being pursued. It involves, as anyone familiar with variety-testing will recognize, a great deal of experimental work, but it is a piece of work which the committee are peculiarly suited to carry out, for, being in touch with the National Association of British and Irish

[1] Since this *résumé* was written baking trials have been completed on Fife grown in England, Browick, Garton's New Era, and on three sets of Rivet. The results from the three first named sorts disclose no substantial difference due to the stage of ripeness at cutting. From the sets of Rivet varying results were obtained, so that the only positive statement which can be made on this point is that Rivet is the only sort tried which has shown under any conditions a gain in strength resulting from cutting at an early stage of ripeness.

Millers, and through them with the wheat markets of the world, they are able to obtain numbers of varieties otherwise unobtainable.

The results which have already accrued are full of interest, both to the practical man and the scientist, for whom they open up a number of complex physiological problems. The general plan of the experiments was to test the strength of various wheats as imported, and then sow them as nearly as possible under uniform conditions. Some of the results only can be quoted. One of the first commercial wheats to be tested was that known as Hard Kansas, a wheat, which, unlike the majority met with in commerce, was found to consist practically of one variety only, presumably Turkey, and not a mixture of all the varieties grown in a district. This was grown alongside Carter's Stand Up in 1901. The latter yielded 44 bushels per acre, weighing 64 lbs. per bushel, the Kansas 32 bushels, weighing 67¼ lbs. The following season the yields were about 30 bushels per acre in each case. The bearded ears, the weak straw and extreme susceptibility to rust were all against Kansas from the farmer's point of view, but in the mill it proved as was then considered satisfactory. It was so hard that when ground by itself on a complete roller plant the feed of the mill had to be reduced to give a good "clean up." In the bakehouse it was far superior to average English wheat, and it earned 75 marks. The following season, 1902, its strength was again indicated by 75, but as it fell away to 65 in 1903 the further cultivation was abandoned.

Several of the best of the Hungarian wheats were obtained by Mr Gyorgy, now Minister of Agriculture in Hungary, for testing in the same manner. Two of these, Tisra Videki and Feherinegyei, as imported, proved so extraordinarily strong that marks of over 100 each had to be assigned them. These were grown in 1904, but in spite of the favourable season their strength deteriorated to such an extent that they were little better than ordinary English. The Tisra Videki fell from 120 to 73, and the Feherinegyei from 112 to 77 marks. Two other varieties, Banati and Diozegi, originally marked at 80, retained their strength satisfactorily. In the case of the first two it is clear that the conditions under which they had been grown on the Hungarian plains were largely responsible for these abnormally high marks. Thanks again to the kindness of Mr Gyorgy the committee were able to extend this experiment further by growing Square-Head's Master for two seasons, under the conditions which produced the strong Tisra Videki. A quantity of the grain was sent over here for baking trials, but even without these it was evident that it was simply a good

sample of Square-Head's Master with no abnormal strength about it, and that the climatic conditions had not modified it appreciably. The baker marked it at 70, so confirming this opinion. A supply just received, grown in 1905, for the third year in Hungary, indicates the same result.

Many of the wheats shipped here from Russia are notoriously strong, and in consequence numbers of these have been tested. One unusually good sample, marked 100, consisted of two lots, one grown near Odessa, the other in the Dnieper district, both on light sandy land where the yield was from 12—14 bushels per acre. As grown under English farming conditions the yield went up to 26 bushels, but the crop had nothing to recommend it; the straw was weak and the strength had dropped to 70. Among the many varieties present in the crop were two which were isolated as they appeared to possess unusual strength. One of these is a bearded yellow, the other a bearded red-chaffed wheat; the varieties have still to be properly identified. Pure cultures of these have been raised and sufficient of the yellow-chaffed one obtained for a baking test in which the early opinion as to its strength was justified by its earning 90 marks. Further baking trials of it still have to be made, but after four years' cultivation here it appears to the eye to be as good as when first detected. The red-chaffed one has recently for the first time been baked and it too possesses great strength. In addition its straw and its cropping power are fairly satisfactory, and it may prove worthy of extensive cultivation. Besides these some hundreds of samples of strong Russian wheats have been sown. The majority have proved to be rapidly growing wheats with poor cropping capacity and grain of only medium quality. A few varieties believed to be of more than ordinary strength have, however, been found among them, and these are being kept under observation at present.

The wheats from the north-west of the United States have been tested in the same manner. These again are graded wheats or mixtures of all or nearly all the varieties of a district. On sowing a commercial sample of such a wheat, No. 1 Northern Duluth for instance, several distinct varieties were found in the crop. Some of these varieties multiplied more rapidly than others, so that the composition of the crop from year to year changed and consequently its baking marks would in a very few years have been of no value as indicating whether any of the sorts comprised in the sample were worth further testing. Four of the best varieties were picked out in 1903 and cultivated

since in a pure state, in order to get a sufficient quantity for field trials.

The investigation of the Manitoban wheats has been carried out for the most part on pure varieties instead of commercially-graded seed. In 1902 Dr Saunders sent from the Ottawa Experimental Station quantities of Improved Fife, Preston and Percy. These were grown at Wye and the seed distributed to several agricultural institutions for trial as spring- and autumn-sown wheats. In all these cases the strength of the home-grown progeny was satisfactory, ranging as the result of at least twenty baking tests from 73 to 88 on diverse soils. Of the three it was evident that the Fife was the most suitable for English conditions. Preston and Percy may be more valuable in Canada, where early ripening is of more importance than with us. Further trials both as spring- and autumn-sown wheats have shown that Fife is retaining its strength well, the bakehouse tests marking it from 75 to 89. If only its yielding power were more uniformly satisfactory the cultivation of this variety would go a long way to solving the committee's problem. As it is, Fife has been found to give good crops on some soils. In one case when grown alongside Golden Drop it yielded 43¾ bushels per acre, against 41½ for the latter. Yields of 30 bushels and over have not been unusual in the various trials, but on some lands, more particularly heavy ones, more than 25 bushels cannot be relied on. In the coming season it is being tested by many growers in all parts of England, and it should then be possible to indicate where the cultivation of Fife will prove profitable.

The question now arises as to whether, presuming Fife is suitable for English cultivation, it will be necessary to import seed yearly from Canada, or whether its strength will be retained indefinitely. So far it has been tested for four seasons and there appear no symptoms of any falling away in this respect. Further, there is evidence to show that no deterioration need be expected even under our climatic conditions. Part of this evidence turns on the interesting announcement recently made by Dr Saunders that Fife is none other than Galician wheat, a variety well known in parts of the Continent. Some few berries of Galician found their way in a bulk of seed to David Fife (or Fyfe) in Canada, and proving on account of their superior hardiness to be the sole survivors of the sowing he propagated them further. From these few plants the whole stock of Canadian Fife appears to be descended. Galician wheat can readily be obtained in Western Europe grown under climatic conditions very similar to our own, and

it still appears to have all the strength of its Canadian descendants. Another unlooked for proof that its strength is not a diminishing quantity has been found. Mr Goodwin, of Kidderminster, informed the committee that in his neighbourhood a wheat locally known as Cook's Wonder was being grown which he believed to be Fife, or some closely related variety. He provided them with four samples, grown on widely different types of soil, which on baking proved to be really strong. On inquiring further into the matter it was found that this wheat had been brought over from Canada in 1892 and that no further importation had been made. It has therefore retained its characteristic strength under our climatic conditions for thirteen seasons.

If one must summarize the most important facts brought out in these extensive variety trials the first is that while the quality of some wheats may change enormously with climatic and soil conditions, there are other varieties in cultivation which retain their strength under all conditions. Fife, for instance, is strong whether grown in England, Canada, the United States, W. Europe, or Australia.

It is a peculiarity of certain wheats, such as Fife, to retain this characteristic, whilst others depreciate. So far there are no means of telling how a variety will behave without actually cultivating it, and resorting to either the miller's judgment or the bakehouse. Next in importance is the fact afforded by the series of Lammas and Square-Head's Master trials, that given two varieties of unequal strength, no matter what the soil conditions are, the one which proves the stronger on one soil will prove the stronger on all.

Now that the proof has been given, more definitely than could have been hoped for considering the time the experiments have been in progress, that there is no difficulty in growing strong wheats in this country this knowledge has to be used to the fullest advantage. If the cultivation of Fife wheat, as is probable, should prove unprofitable in many situations, what substitute can be offered for it? Of all the pure strains, now isolated and under observation, numbering nearly a hundred, it would be rash to predict that any are absolutely suitable for extended cultivation. Some possibly surpassing Fife in strength give no promise of a paying yield, others which crop better have thin reed-like straws, and many of them are so extraordinarily susceptible to the attacks of yellow rust that it is problematical whether they would not die out altogether under our conditions.

The attempts made to solve the original problem of combining high-

yield with strength have to be described now that it has clearly come within the bounds of possibility. To achieve this two methods have been resorted to, one the process described by plant breeders as selection, the other hybridizing. In the case of the former the best plants with regard to yield and strength have been chosen from a pure culture of one of the strong varieties, so that strength instead of yield was assured from the first. A portion of the grain from the best five or so of these has been sown in order to obtain twenty-five plants descended from each, and from the small plot producing the best crop the best plants are again chosen, and the process repeated. The variability of the yield appears to be very slight, for after four seasons no manifest improvement has resulted. The attempt to select a strong wheat from such a high-yielding wheat as Rivet hardly appears to be worth making for its endosperm characters are singularly constant. Such an experiment is, of course, not a final one, and it might well be carried out on a larger scale at stations having greater facilities for such trials than are at the disposal of the committee. The data obtained would be valuable, especially as the chances of complications arising through vicinism are remote. The hybridizing experiments however offer far more chance of success in a comparatively short time. These were commenced in 1901[1], and the task of investigating the inheritance of all the numerous characters shown by the different varieties of wheats was undertaken. This seemed essential as it was clear even then that a considerable number of wheat varieties would have to be crossed together. As already stated, the many varieties which have been selected for strength are frequently far from satisfactory in other respects. By applying the experience gained from these preliminary breeding experiments new varieties can be built up in which the objectionable features are wanting, whilst satisfactory ones are retained. At present there is, and naturally so, a great deal of scepticism as to the fixity of such hybrid varieties, and under these circumstances it is satisfactory to be able to state that where the hybrids already raised have been grown in plots containing several thousand individuals they have proved as true to type, level, and uniform in ripening as the oldest varieties in cultivation. The difficulty of obtaining fixed types without endless "rogueing" has practically disappeared.

To build up varieties of wheat suitable both to farmer and miller it was essential to study in great detail the inheritance of strength. The

[1] Biffen, *Journal of Agricultural Science*, Vol. I. No. 1.

matter was complicated from the beginning by the fact that in some wheats the quality deteriorated rapidly, and also by the fact that seed could rarely be spared for analysis during the critical first and second generations. In consequence the inheritance of this characteristic has been judged by eye: that this is fairly satisfactory is shown by the fact that an expert miller when given the grain of two hybrid varieties was able to recognize in them the characters of their parents, although he had no previous knowledge of their history. By the time the third generation is reached and the hybrid varieties are known to be fixed small quantities of the grain can be spared for analysis.

It would be premature to state without any reservation that strength and "weakness" form a pair of Mendelian characteristics, but the assumption that they are so has proved a very valuable one in building up desirable varieties. Each season a number of the best of the strong varieties already selected have been crossed with suitable high-yielding parents, and a number of series of hybrid varieties are being raised. Ruthless selection is practised, and unless wheats prove satisfactory in all features they are destroyed at once, exceptions only being made where they appear of possible value as fresh parents. So far about 40 types for the most part of Fife parentage have survived the ordeal, and these are now being cultivated in the open to determine their yielding-power. They are somewhat diverse in habit, some being loose, others square in the head, some with white, others with red grain, etc., but in each case, as far as the eye can judge, the strength of the parent Fife has been maintained.

THE LAW OF SEQUENCE IN THE YIELD OF WHEAT FOR EASTERN ENGLAND. 1885—1905[1].

By W. N. SHAW, Sc.D., F.R.S.,
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In February 1905 I made a short communication to the Royal Society of London (*Proceedings*, Vol. LXXIV. page 562), upon an apparent relation between the yield of wheat for England in the 21 successive years 1884 to 1904 and the aggregate rainfall for the three autumnal months (September, October, November) of the preceding years of the principal wheat-producing districts of Great Britain. The relation was put into the algebraical form:

$$\left.\begin{array}{l}\text{Yield of wheat for}\\ \text{England, per acre}\end{array}\right\} = 39{\cdot}5 \text{ bushels} - \tfrac{5}{4}\left\{\begin{array}{c}\text{previous autumn rain-}\\ \text{fall in inches}\end{array}\right\} \quad (1).$$

In considering the application of the formula to the computation of the yield for the 21 years specified, 7 years were found to give large differences, and reasons were given for regarding the 7 years as being otherwise anomalous.

An endeavour to pursue the investigation of this suggestive relation between yield of wheat and autumn rainfall has disclosed a curious relation between the numbers representing the yield of wheat for a selected part of England, during the last 21 years. This relation is irrespective of any connexion with the previous autumn rainfall, but as it was disclosed in the course of the investigation of that connexion and may ultimately prove to be associated therewith, I will set out the figures first from that point of view.

[1] The earlier part of this paper is a reproduction with slight modifications of a paper on "The Law of Sequence in the Yield of Wheat for Eastern England, 1885–1904" contributed by the author to the "Hann Band" of the *Meteorologische Zeitschrift*. Vieweg und Sohn, Braunschweig, 1906.

The formula already referred to in the *Proceedings* of the Royal Society was based on figures against which objection might be raised, on the ground that they were not strictly correlative. The wheat values were the averages for England; the rain values the averages for those districts of Great Britain where wheat is grown in considerable quantity. It is not easy to obtain figures which refer to an area sufficiently large to eliminate accidental local influences and are, at the same time, properly correlative; but I endeavoured to obtain correlative figures by taking the rainfall values for the district "England East" of the *Weekly Weather Report* and compiling the corresponding values of the yield of wheat from the results given in the official returns of the Board of Agriculture for the counties included in the district.

These figures are, so far as I know, quite unexceptionable for the purpose of the comparison in view. They are doubtless subject to incidental errors, but both on the side of rainfall and on that of yield they are homogeneous for the period to which they refer. It is a matter of regret that they do not extend beyond twenty-one years, the limit set by the wheat returns. The figures for rainfall and for yield of wheat are given in a table at the end of this paper.

The subsisting relation between the autumn rainfall and the wheat-yield of the subsequent year is sufficiently evident, though the constants are different from those in the equation (1) already quoted. For the Eastern Counties the variation of yield in the twenty years is from 25·2 bushels per acre in 1893 and 1904 to 36·3 in 1898, and the formula

$$W = 46 - 2{\cdot}2\,R \qquad (2)$$

gives the yield within the limit of accuracy of 2·1 bushels per acre for thirteen years out of the twenty-one including 1905; but there are eight exceptional years when the yield computed by the formula differs from the actual yield by more than 2·1 bushels. The exceptional years are not all the same as those which were exceptional in the comparison for the whole of England, and on two occasions in particular, viz. 1886 and 1903, the differences, one in excess, the other in defect, are very great.

In the previous comparison an explanation of the differences in exceptional years was given that was *primâ facie* reasonable, and I examined the figures for the East of England in the hope of finding a similar explanation for the differences. In such an enquiry one naturally regards the rainfall data as fundamental, and looks for an explanation of the deficient or too abundant harvests in the accidents that may occur to the crops from floods, hailstorms and other causes,

because the damage done by such accidents bears no numerical proportion to the quantity registered by a rain-gauge. When I plotted diagrams representing the two quantities, rainfall and yield, I expected to find marked irregularities in the yield diagram, with no counterpart in the rain diagram; and I was, for that reason, the more surprised to find that the yield diagram exhibited a singular regularity of sequence, while it was the rain curve that had what may be called abnormal irregularities.

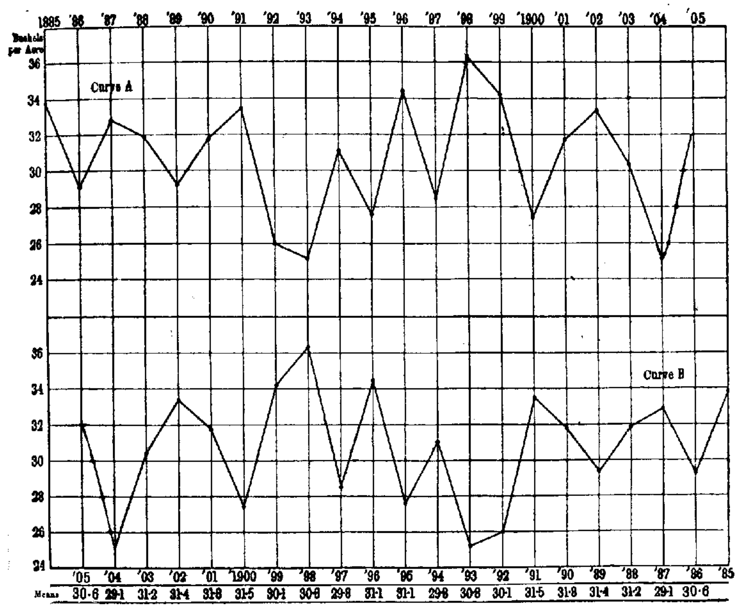

FIG. 1.

The most singular point about the regularity of sequence of the yield diagram is that the curve is almost perfectly reversible with reference to the epoch 1895–6. The average yield for the two years, 1895, 1896, is (31·1 bushels) almost exactly the same as the average yield for the twenty-one years (30·9 bushels); thus 1896 compensates for 1895, and in the same way 1897 compensates for 1894, 1898 for 1893, and so on, without any really considerable exception throughout the twenty-one

years. The worst case of all, an exceptionally bad one, is that for 1887–1904, which, however, gives a mean differing by less than 2 bushels from the average for the twenty-one years.

Figure 1 represents this curious coincidence. Curve A shows the yield of wheat for England East in successive years, and curve B shows the yield *for the same years taken in the reversed order.* It will be seen that curve B is very similar to the simple reflexion of curve A from the base line. The numbers at the foot of the figure show the mean value of the ordinates of the two curves in bushels per acre. If the curve B were a perfect reflexion of curve A, all the mean values would be identical, and it will be seen that they are very nearly so. They range between 29·1 for the pair of years 1887–1904, and 31·8 for the pair of years 1890–1901. The average for the whole twenty-one years is 30·9, so that the greatest difference of the mean of any of the pairs of years from the average of the twenty-one years is 1·8 bushels per acre in the one direction, and 0·9 in the other.

The reversal of the curves can be more strikingly exhibited if one of them be traced and the trace turned round and superposed on the original.

It seems scarcely possible that this compensation, persisting through so many years, should be fortuitous. It would be accounted for if the figures representing the yield of wheat were points on a periodic curve of complex periodicity such that a number of important components concurred in a nodal point in the epoch 1895–6. The test for a nodal point between 1895 and 1896 would be that the ordinates should be reversed in the way in which they are very approximately reversed in actual fact.

It is surprising that this evidence of periodicity exhibits itself in the wheat values, while it is certainly not conspicuous in the autumnal rainfall. The years that are exceptional as regards the rule of parallelism between the autumn rainfall and yield of wheat, viz. 1886, 1889, 1896, 1897, 1901, 1903, and 1905 do not appear as exceptions to the rule of reversal of the wheat yield with reference to 1895–6. The real value for the yield in 1903 is 6·2 bushels below the amount calculated from the autumn rainfall formula, but it compensates the yield for 1888 quite satisfactorily. The only year which shows considerable divergence from the rule of compensation is 1904, which agrees very well with the rainfall formula, but has too small a yield to compensate 1887. It will be seen from the sequel that the defect may probably be attributed to the 1887 yield.

Assuming from the reversal with reference to the epoch 1895–6 that the yield can be represented by a series of periodic components concurring in a node at that epoch, I have endeavoured by an examination of the figures to determine the period and amplitudes of the component oscillations.

There is, so far as I am aware, no organised method of doing this, except for the case of periodic variations of known period with harmonic components and a large number of ordinates for a complete period. In the present case the fundamental period could not be regarded as known. A careful examination seemed to show that the curve might contain components of 2 years', $3\frac{1}{2}$ years', and 11 years' period, and the combination would then only recur completely in 154 years.

To deal with complex periodic variations of unknown period we can proceed by the addition of ordinates with a fixed interval, and so eliminate variations of a definite period[1].

Thus if one of the component variations be

$$a_n \sin \frac{2\pi}{n} t,$$

where n is the period in years, t the time in years from a node, adding the ordinates with an interval of m years gives for this component in the secondary curve the sum,

$$a_n \sin \frac{2\pi}{n} t + a_n \sin \frac{2\pi}{n} (t+m),$$

and therefore a resultant of the same period,

$$2a_n \cos \frac{\pi m}{n} \sin \frac{2\pi}{n} \left(t + \frac{m}{2}\right) \quad \text{.....................(3).}$$

The amplitude is zero if $n = 2m$, and thus, in a curve of complex periodicity, the component $n(2m)$ is eliminated by the addition of ordinates with intervals of m years, while the amplitudes of all the other components are altered by the factor $2 \cos \frac{\pi m}{n}$; the phase of each is put forward by $\frac{m}{2}$ years.

This process has been applied to the curve of wheat-yield by taking the mean of *consecutive ordinates* whereby the variation of two years'

[1] I owe this method of dealing with complex periodic variations to Professor G. Chrystal of Edinburgh, who uses it for the discussion of the complex oscillations of the water of lakes under the name of the method of residuation.

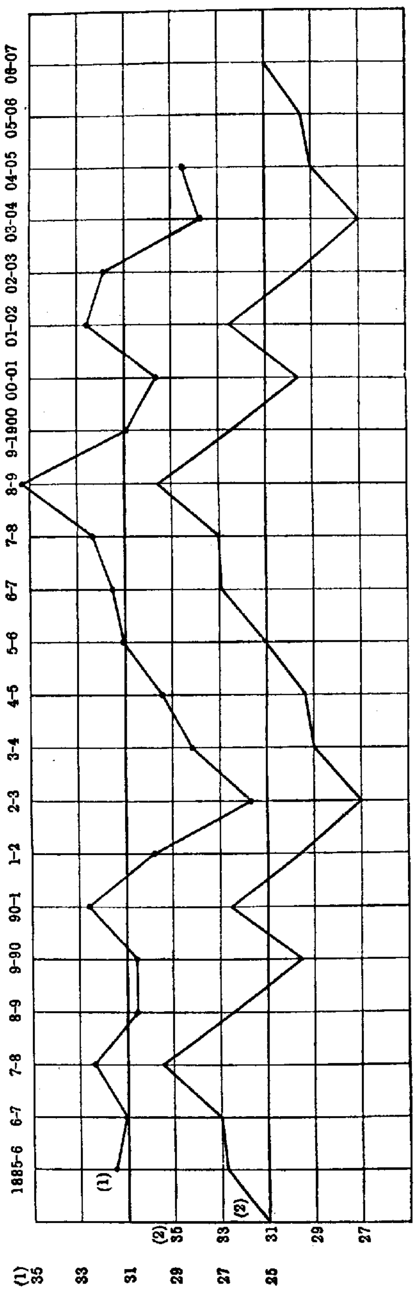
1885-6
6-7
7-8
8-9
9-90
90-1
1-2
2-3
3-4
4-5
5-6
6-7
7-8
8-9
9-1900
00-01
01-02
02-03
03-04
04-05
05-06
06-07
(1)
(2)
(1) 35
33
31
29
27
25
(2) 35
33
31
29
27

Fig. 2.

period is eliminated; the variations of other periods are put forward in phase by half a year, and the amplitudes are all altered as indicated by the coefficient. The result, which is represented in the upper curve (1) of Figure 2, shows a very considerable simplification of the original curve of Figure 1.

Further procedure by this process is not practicable for two reasons. First, we have only a single point for any year and no means of satisfactory interpolation. We can, therefore, only eliminate components of an even number of years by addition of ordinates. Secondly, if there be any accidental errors in the curve they get distributed over the sum in such a manner as to add to the complexity. There is a considerable reduction of the variation of ordinates upon taking the means of the ordinates (1) + (6) and also of (1) + (7), which points to a component of about ten or twelve years' period. Taking this variation I have endeavoured to guess the components of curve (1), Figure 2, by an inspection of the curve itself.

I have obtained in this way the periodic curve from which the ordinates of Curve (2) are taken and of which the equation is:

$$y = 31 + 2{\cdot}8 \sin \frac{2\pi}{11} n + 0{\cdot}4 \sin \frac{4\pi}{11} n - 1{\cdot}2 \sin \frac{6\pi}{11} n + 1{\cdot}2 \sin \frac{8\pi}{11} n,$$

where n is the number of years (positive or negative) from the node at 1895–6. The successive ordinates correspond with the substitution of $n = 1, 2 \ldots$ in this equation.

The agreement between the curve constructed from this formula (Curve 2) and the original (Curve 1) representing two years' means of actual yield is remarkable. For the first four points the calculated yields certainly exceed the observed, and by an amount which reaches 3 bushels in 1887–8, but from 1889–90 to 1904–5 the mean value of the deviation between observed and calculated values, without regard to sign, is only ·67 bushels, and the maximum deviation + 2·3 bushels for 1902–3. The mean difference between the two curves is 0·1 bushel per acre. A shift of the curve through the space representing ·1 bushel would, therefore, slightly improve the correspondence. The representation of the real curve by the calculated one is, however, already as complete as one can expect any calculation of this sort to be, and pending the compilation of further data we may consider the average yield of wheat for successive two years to be effectively represented by a simple periodic variation of eleven years, with subsidiary harmonic components of 11/2, 11/3, and 11/4 years.

The original article in the Hann volume continued as follows:—

"It is evident that if this representation is accurate the average yield for years to come should fall in with the same formula; in other words the formula allows us to predict the yield of future years. The prediction for 1905 on this basis is a yield that will give with 25·2, the yield for 1904, a mean for the two years of 29 bushels. In other words, the yield for the Eastern Counties for 1905 should be 32·8 bushels. The rainfall calculation for the same year gives 37·6 bushels. There is no doubt that the yield is large, but the Official Report has not yet been issued, so the precise figures cannot be given. If 37·6 proves to be the correct figure there will be rather a large error (2·6 bushels) in the calculated harmonic curve."

The actual figure for 1905 turns out to be 32·0, so that it agrees better with the continuation of the composite curve than with the calculation from the autumn rainfall.

Since the article for the Hann volume was completed, I have extended the computation so as to represent the sequence of the yields for individual years by the sum of a series of six harmonic components of the eleven years' period. This extension of the reasoning accounts for the two year oscillation (which was eliminated from Fig. 2 by taking the average of consecutive years) and regards it as a 'beat' between the oscillations of the fifth and sixth components of the eleven years' period. According to this analysis, of which the details are given in a paper read before the Royal Society on May 17, 1906, the sequence of individual yields for the twenty-one years, 1885–1905, is represented with remarkable fidelity as the sum of six harmonic components of the eleven years' period, each with a node at the epoch 1895–6. The amplitudes of the several components are as follows:

Period............	11	$\frac{11}{2}$	$\frac{11}{3}$	$\frac{11}{4}$	$\frac{11}{5}$	$\frac{11}{6}$	years.
Amplitude [31]	+ 2·9	+ 0·5	− 1·8	+ 2·8	+ 1	+ 1	bushel per acre.

The − sign indicates a descending node.

Values derived from the addition of these six components are given in the second column of the Table on p. 28, so that they may be compared with the actual values given in the third column. It will be seen that the direction of change from year to year is correctly given in every case and the agreement between the computed and the actual values is very close, except for the years 1887 and 1888, when the actual yield is too small and 1903 when it is too large. The way in which the minor fluctuations in the yield are indicated in the computed curve is very remarkable when it is considered that the components all refer

to an eleven years' period or its submultiples, and the comparison extends over 21 years and not merely eleven years.

If the representation of the actual values by the computed curve have any real counterpart in the law of sequence of the yield of wheat it follows that the yield should repeat itself after eleven years. This is a result which is easily tested. In arranging the Table on p. 28, I have put down not the actual yields but the excess or defect, + or −, from 30·8 bushels, and have grouped together the pairs of years with eleven years' interval; thus 1885 and 1896 are together, 1886 and 1897, and so on. The comparison between any year and the one eleven years later can thus be easily made. For three of the pairs of years out of the ten represented in the Table the yield is repeated within a tenth of a bushel, for six it is repeated within a bushel. In every one of the ten pairs the yields are either both in excess or both in defect. The three cases where there is a substantial difference between the yields for a pair of years eleven years apart are those which contain the years 1887, 1888 and 1903 already mentioned.

That this simple principle of repetition after eleven years, which is easily traceable in the curves of Fig. 1, should not have been noticed until after a somewhat lengthy discussion is due principally to the fact that the yields for 1887 and 1888 are both substantially smaller than the computed values, and the curve, which should have had a salient point then, as it has in 1898 and 1899, has no conspicuous prominence, although its shape is not dissimilar in detail from the corresponding part eleven years later. It is curious, however, that in both the years named the yield, although too small from the point of view just mentioned, exceeded that computed from the previous autumn rainfall, so that the explanation of the defect, if indeed there be one discoverable, is rather recondite. The point is interesting because we have again in the current year a case in which the indication from the autumn rainfall is in the opposite direction to that from the principle of repetition after eleven years, and we do not yet know how it will be resolved[1]. It raises the question of the real meaning of the relation between the autumn rainfall and the wheat-yield of the subsequent year.

A paper by Mr E. Mawley (*Quarterly Journal Roy. Met. Soc.*, Vol. XXIV. p. 75, 1898) "On weather Influences on Farm and Garden Crops" suggested to me that a small autumn rainfall might be associated with a small summer rainfall following, and might therefore become an index

[1] NOTE. December, 1906. The official estimates for the several counties are not yet published. The general opinion is in that the yield was large but irregular as regards the Eastern counties.

of general meteorological conditions rather than itself a determining cause of the amount of the future crop. To illustrate this point I have included in the Table on p. 28 in the same line with the figures for the crop for each year the excess or defect from the average of 20 years of the rainfall of each season, from the autumn before to the summer of the gathering of the crop. I have also added as an index of temperature-conditions the "accumulated temperature above 42° F." for the same seasons. The figures are taken from a paper on "The seasons in the British Isles" in the *Journal of the Royal Statistical Society*, Vol. LXVIII. Part II. (June 1905).

Then at the foot of the Table, following Mr Mawley's plan, I have given the average results for the five *best* wheat years and the five *worst*. The result is remarkable. Not only is the autumn rainfall deficient for the good years and excessive for the bad, but, with the exception of the accumulated temperature for the summer, which is above the average in both cases, the character of the season for the good years is the opposite of the corresponding season for the bad years in every case. Judging by the average results for the five years a good wheat yield is preceded by a dry and warm autumn, a rather dry and warm winter, a rather wet and cold spring, and a dry and slightly warm summer, while a bad wheat year has, on the other hand, a wet autumn of average temperature, a wet and cold winter, a dry and warm spring and a moist warm summer.

This bears out the suggestion that the autumn rainfall is in a way the key to the subsequent seasons, but it still remains to consider whether these results are merely average results for the five selected years or whether they apply to individual years. Looking down the figures for the individual years it is clear from the juxtaposition of the signs that a wet autumn usually means a deficient crop and *vice versa*; but it is also clear that a wet autumn is usually associated with a relatively dry spring and *vice versa*, as is indicated in the averages for the five years. On sixteen occasions out of the twenty-one a deficient autumn rainfall has been followed by excess of rainfall in the spring or *vice versa*. On three other occasions the spring rainfall has been normal or within a tenth of an inch of it, leaving only two exceptional occasions, forming the pair 1886 and 1897, when the heavy autumn rainfall was succeeded by spring rainfall above the average. On the other hand, on sixteen occasions out of the twenty-one the deviation of the winter rainfall from the normal has been in the same direction as that of the autumn rainfall. There appears to be no numerical relation between the amounts of excess or defect in either

case, but it does appear that the suggestion of the five year averages that a dry autumn is followed by at least a rather dry winter and a rather wet spring and *vice versa* is borne out in the large majority of cases.

As regards summer rainfall or accumulated temperature, however, there is little information to be got from the figures. Good wheat years are as a rule preceded by warm winters, and bad years by cold ones, but the figures are very irregular. Such a pair of years as 1888, 1899, as a glance at the figures will show, seems to make any generalisation about the relation of the temperature to the wheat or the other elements hopeless from the first.

There are, however, sufficient indications of underlying connexions to encourage further investigation. The eleven years' periodicity in the yield, with its consequence, the repetition of values after eleven years, seems to be the most directly applicable of them. It would of course be unwise to regard the law of sequence thus indicated as definitely established.

The data available give us only approximately two eleven year periods, so that we are not able to apply an adequate test to the suggestion that the curve is in reality a curve of eleven years' period with its harmonic components. Many circumstances besides those associated with the practice of farming might be found to interfere with its persistence. It is possible that the concurrence in a node at the epoch 1895–6 is not mathematically strict; it is possible that the components of about four years', three years', or two years' period are not, strictly speaking, harmonics of the eleven year period. They may disclose increasing divergencies as the years accumulate. All that we can say is that for the twenty-one years for which data exist the representation of the values by a curve of eleven years' period is singularly close to reality, especially for the years 1889 to 1905. Further data, or perhaps data for some other quantity than wheat, may disclose a more definite result. In the meantime it is worthy of note that on the average for the seven counties comprised within England East, the good wheat years have occurred with the following intervals from 1885[1] (itself a good year)

$$\left\{\begin{matrix} \text{2 years,} & \text{1 year,} & \text{2 years,} & \text{1 year,} & \text{3 years,} & \text{2 years} \\ 1887^* & 1888^* & 1890 & 1891^* & 1894 & 1896^* \end{matrix}\right\}$$

completing eleven years, and then again

$$\left\{\begin{matrix} \text{2 years,} & \text{1 year,} & \text{2 years,} & \text{1 year,} & \text{3 years} \\ 1898^* & 1899^* & 1901 & 1902^* & 1905 \end{matrix}\right\}.$$

[1] The years marked * are those which according to the computed curve give yields exceeding the average by at least 2 bushels; for the others the theoretical yield is not much above the average.

As this order of succession has now occurred twice, it seems worth while to consider the question in relation to the rotation of crops.

TABLE.

Year	Yield above or below 30·8 bushels per acre computed from six harmonic components of eleven years' period.	Actual Yield above or below 30·8 bushels per acre.	Rainfall in inches above or below the average of the 20 years 1881–1900				Accumulated Temperature above 42° F. in day degrees above or below the average of 20 years, 1881–1900			
			Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer
1885	+4·5	+3·0	−0·3	+1·3	+1 0	−3·2	+25	+46	−104	−89
1896	+4·5	+3·6	−0·9	−1·9	−0·2	1·4	+113	−4	+33	+63
1886	−0·7	−1·6	+5·0	−0·6	+0·6	−0·9	−146	−86	−49	−60
1897	−0·7	−2·3	+1·9	+2·5	+0·1	−0·9	−72	−25	+14	+110
1887	+4·8	+2·1	−0·7	+0·3	+0·2	−3·0	+221	−49	−116	+163
1898	+4·8	+5·5	−2·5	−1·2	+0·5	−1·6	−58	+71	−68	+2
1888	+4·7	+1·1	−0·9	−1·7	+0·4	+3·4	−244	−81	−87	−213
1899	+4·7	+3·4	−2·4	−0·4	+0·7	−2·8	+244	+139	−21	+201
1889	−2·0	−1·5	−2·2	−1·6	+2·0	+1·8	−40	−46	+67	−25
1900	−2·0	−3·3	+0·2	+3·0	−1·2	+0·2	+99	−36	102	+167
1890	+0·2	+1·0	−0·7	−0·7	0·0	+1·5	−68	−1	+5	−119
1901	+0·2	+1·0	−2·8	−0·4	+0·2	−2·7	+112	+43	−45	+71
1891	+2·4	+2·7	−2·0	−2·6	+1·0	+1·3	+122	−9	−143	−96
1902	+2·4	+2·6	−2·8	+0·4	+0·4	+0·9	+79	+8	−51	−121
1892	−4·3	−4·8	+0·6	−0·2	−0·3	+1·9	+56	−20	+35	−147
1903	−4·3	−0·4	−3·3	0·8	+0·5	+5·0	+37	+128	+34	−85
1893	−4·4	−5·6	+2·6	+0·6	−3·7	−0·6	−153	−28	+260	+179
1904	−4·4	−5·6	+1·4	+0·7	−1·0	−1·6	+19	−76	+26	+91
1894	+1·1	+0·3	−0·7	−0·5	+0·8	+1·1	−28	+44	+36	−67
1905	+1·1	+1·2	−3·8	−1·4	0·0	−1·2	−6	−11	+19	+187
1895	−4·1	−3·1	+0·3	+0·4	−0·7	+1·4	−27	−69	+62	+15
1906	−4·1	?	−1·6	+1·2	?	?	−127	−20	?	?
Five best wheat years		+3·6	−2·2	−0·8	+0·6	−1·5	+89	+49	−61	+16
Five worst wheat years		−2·5	+1·0	+0·9	−1·4	+0·3	−1	−46	+56	+85

NOTE ON AN APPARENT SECULAR CHANGE IN THE ROTHAMSTED DRAIN GAUGES.

By EDWARD J. RUSSELL, D.Sc. (Lond.),
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DR MILLER'S interesting and important paper in the last number of this *Journal* (Vol. I. p. 377) raises several points in connexion with the long-continued action of rain on uncultivated and uncropped soils.

Some of the water falling on the drain gauge is retained by the soil, some evaporates, and the rest percolates, but over a series of years the amount of percolation is correlated mainly with the amount of evaporation, when the latter is high the former is low, and *vice versâ*.

Evaporation takes place mainly, but not entirely, from the surface of the soil. A certain amount must go on from the individual particles in the interior of the soil, and the water vapour will diffuse out from the pore spaces at a rate greater than that at which air diffuses in. Other things being equal, the greater the depth to which air can penetrate and from which water vapour can pass out by diffusion, the greater the amount of evaporation. Thus we should expect the greatest evaporation from the 60 inch gauge, a smaller amount from the 40 inch gauge, and the least from the 20 inch gauge.

Examination of the figures for the first four years shows that this expectation is realised.

TABLE 1.

Evaporation for period 1870–1874.

20 inch gauge	40 inch gauge	60 inch gauge
69·51	71·04	78·90

Calculated as percentages on the evaporation from the 60 inch gauge,

88·10	90·05	100

For subsequent years, however, the relationship does not hold.

The total amounts of evaporation in consecutive seven year periods are set out below,

TABLE 2.

Evaporation during 7 year periods.

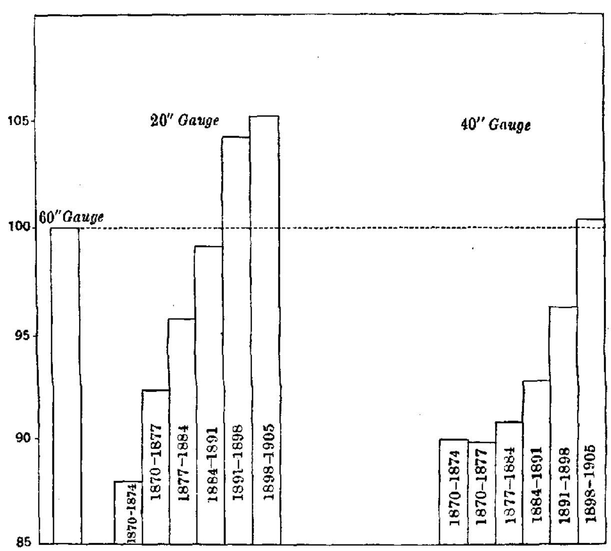
	20 inch gauge	40 inch gauge	60 inch gauge
1870–1877	125·74	122·25	135·81
1877–1884	107·70	102·12	112·39
1884–1891	98·30	92·01	99·10
1891–1898	98·76	91·26	94·80
1898–1905	99·24	94·79	94·23

Calculated as percentages on the evaporation from the 60 inch gauge,

1st 4 years, 1870–1874	88·10	90·05	100
1st 7 years, 1870–1877	92·60	90·02	100
1877–1884	95·85	90·86	100
1884–1891	99·20	92·85	100
1891–1898	104·2	96·27	100
1898–1905	105·3	100·6	100

The percentage results are plotted in the accompanying diagram.

Evaporation from the Drain Gauges, 7 year periods.



It is quite evident that the relationship between the amounts of evaporation from the three gauges is not constant, but shows a regular and progressive change. At first evaporation from the 20 inch gauge

is lowest; it increases, and soon becomes greater than that from the 40 inch, after about 20 years it equals, and finally exceeds, that from the 60 inch gauge. The 40 inch gauge shows the same change relative to the 60 inch, but with a lag of about 14 years. Up till about 1884 no change took place, and the two gauges behaved precisely as, on the above reasoning, one would expect them to do. Then the relative evaporation from the 40 inch gauge began to increase, and during the last 7 years the amount of evaporation equalled that from the 60 inch gauge. It seems safe to predict that in a few years' time the 40 inch gauge will lose more by evaporation than the 60 inch.

Several causes may operate in bringing about this change.

Rain tends to wash the finest particles downwards into the soil. In pasture land this tendency is counteracted by worms and in arable land by cultivation, but in the drain gauge neither of these factors comes into play, the finest particles wash downwards unchecked, and in time may wash out of the gauge altogether. As they disappear the soil becomes more and more permeable to air and water vapour, evaporation can take place to a greater extent than before, the gauge tends to become dryer, and when rain comes, more of the water is held back and less percolates. Naturally this change is seen in the 20 inch gauge long before it appears in the deeper ones.

It may be objected that the Rothamsted soils contain about 20 per cent. of clay, and the three tons of water falling annually upon the gauges could not possibly wash out anything like this amount. We are, however, not concerned with the total clay but only with the unflocculated portion, which though unknown, is probably small.

The oxidation of the organic matter in the soil of the gauges would operate in the same way. This process is continually going on, it results in the formation of empty spaces into which air can pass and from which water vapour can diffuse. This change, like the other, becomes apparent in the shallow gauge before it does in the deeper.

Evaporation and drainage are complementary. If the above hypothesis is well founded, it explains why the drainage through the 40 inch gauge is on an average more than through the 60 inch or the 20 inch; it also explains why the 20 inch gauge at first yielded more, and afterwards less, drainage than the 60 inch, and why the 40 inch is starting to behave in the same way. On this view the present agreement between the 20 and 60 inch gauges is accidental and temporary, whilst the difference shown for so many years between the 40 and 60 inch gauges is no abnormal feature, but a normal one. Of the three

gauges, in fact, the 20 inch is the abnormal one and not (or not until recently) the 40 inch.

Two or three results follow from the above change if it has taken place. The differences in the amount of evaporation from the 20 and 60 inch gauges should be accentuated in the dry months, since air penetrates more readily into, and water vapour diffuses out from, the former than the latter. On the other hand the differences should be less marked during wet months. The drainage from the 20 inch gauge should, therefore, in months of low rainfall be less, and in months of high rainfall more, than that from the 60 inch,—more, because the soil is more permeable, and only its greater evaporative power ever makes the drainage less. The data given on page 382 of Dr Miller's paper completely accord with this expectation.

TABLE 3.

Months of low rainfall, average of 35 *years.*

	Rain	Drainage from 20 inch gauge	Drainage from 60 inch gauge
January	2·29	1·79	1·92
February	1·94	1·39	1·44
March............	1·88	·92	1·00
April	1·90	·50	·53
May...............	2·08	·47	·49
Total	10·09	5·07	5·38

In each case the drainage from the 20 inch is less than that from the 60 inch gauge.

Months of high rainfall, average of 35 *years.*

	Rain	Drainage from 20 inch gauge	Drainage from 60 inch gauge
June	2·41	·65	·64
July...............	2·70	·68	·65
August	2·69	·63	·58
September	2·51	·86	·74
October	3·23	1·83	1·64
November	2·82	2·10	2·01
December	2·52	2·02	2·01
Total	18·88	8·77	8·27

Here the drainage from the 20 inch gauge is greater than that from the other.

These figures relate to the whole period. The monthly values for each separate year up to 1880 are given by Lawes, Gilbert, and Warington in the *Journals of the Royal Agricultural Society for* 1881 (Vol. XVII. p. 330). From these it is evident that the relationship brought out in Table 3 above only begins to hold after the first four years, *i.e.* after the changes in the gauges had begun. During these four years, as one would expect, the 20 inch gauge allows more water to percolate even in dry months than the 60 inch.

Months of low rainfall (Jan.–May) 1871–1874.

Mean rain	Drainage from 20 inch gauge	Drainage from 60 inch gauge
9·646	3·78	3·48

In subsequent years the order is reversed, as shown in Table 3.

A similar, but less definite, relationship is shown by the nitrification figures. Had there been no alteration in the gauges the 60 inch gauge, having the greatest depth and the greatest amount of nitrifiable organic matter, would be expected to yield the largest quantity of nitrates, the 40 inch would give less, and the 20 inch least. If, however, the changes suggested above have taken place, the 20 inch gauge, being now more open to atmospheric influences than formerly, should yield a larger quantity of nitrates than the 60 inch. On the other hand, the 40 inch gauge, having suffered less change, should yield less.

Bearing in mind Dr Miller's caution on p. 390, that "the relation of nitric nitrogen to the quantity of drainage is somewhat complicated," it is nevertheless evident that the nitrates do vary in the manner just indicated.

TABLE 4.

Nitrogen as nitrates per acre in the drainage for 7 year periods.

	20 inch gauge	40 inch gauge	60 inch gauge
1877–1884	40·55	34·69	39·62
1884–1891	32·53	28·84	29·97
1891–1898	29·72	26·58	29·32
1898–1905	29·48	26·55	28·45

Calculated as percentages on the values for the 60 inch gauge.

1877–1884	102·6	87·56	100
1884–1891	108·5	96·23	100
1891–1898	101·4	90·65	100
1898–1905	103·6	93·90	100

The foregoing evidence all seems to point to a secular change in the drain gauges resulting in an increased evaporation of water. It is suggested that the change is brought about by the diminution of organic matter in the soil of the gauges and the action of rain in washing out the finest particles.

SOME OBSERVATIONS ON THE ASSIMILATION OF ATMOSPHERIC NITROGEN BY A FREE LIVING SOIL ORGANISM.—AZOTOBACTER CHROOCOCCUM OF BEIJERINCK.

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SOME years before the appearance of Hellriegel and Wilfarth's work on the sources of nitrogen of leguminous plants, and while the part played by atmospheric nitrogen in the nutrition of crops was under active discussion, Berthelot was making some exact observations on the behaviour of uncropped soils towards the free element. He found that when 50 kilograms of air-dry arable soil were exposed to the air and rain in a vessel for seven months a great increase in the nitrogen content could be observed; the total nitrogen of the original soil had increased from 50 grams to 63, or a gain of over 25 per cent. after allowing for the small amount of combined nitrogen brought down by rain; in another experiment where the soil had first been washed free from nitrates, a gain of 46 per cent. of nitrogen was proved. In many other cases, however, the gain was only from 10–15 per cent. of the original nitrogen present in the soil. Gains have also been observed by many other observers but always much lower than those of Berthelot first mentioned. Berthelot was able to show that the gain of nitrogen in a soil kept in the dark was only half as great as in the same soil exposed to light; while, however, he could observe no increase in the nitrogen of his soil during the winter, Koch has found a considerable increase during that time in soil which was kept in a heap and shovelled over from time to time. Berthelot, observing that when the soil had been previously steamed at 100° no gain of nitrogen followed, concluded that soil organisms must be concerned in the fixation; he was unable, however, to separate by plate methods, a single

one which could assimilate free nitrogen in pure culture. In 1894 Winogradsky published his researches on a bacterium which could fix the free nitrogen of the air while growing in pure culture in a solution containing only dextrose and nitrogen-free salts; this organism he called *Clostridium pasteurianum.* It forms spores, is an anaerobe, and belongs to the group of butyric acid bacteria. The sugar is broken into carbonic acid, hydrogen, butyric acid, acetic acid and butyl alcohol, and for every gram of sugar consumed about 2 mgs. of nitrogen are assimilated from the air. In impure culture directly from the soil up to 3 mgs. of nitrogen are fixed for every gram of sugar decomposed.

During the period between Berthelot's work and the discovery of this organism the view had gained ground that algae growing on and near the surface of soil were able to fix nitrogen directly. In 1888 Frank had observed such a growth on sand exposed to light, and had found that the upper layer showed a considerable increase of nitrogen. In 1892 Schloesing and Laurent proved, both by determining the nitrogen fixed by a soil in a closed vessel, and by observing the diminution of the nitrogen gas in the enclosed air, that a soil exposed to light gains in nitrogen if algae are allowed to grow on the surface, and that the gain is confined to the upper few millimetres of depth. They did not however, employ a sterile soil nor pure cultures of algae. Kossowitsch working with pure cultures of two green algae found no fixation, but observed a considerable increase of soil nitrogen by growing them mixed with soil bacteria. Later Krüger and Schneidewind employing pure cultures of many other *Chlorophyceae* could also find no nitrogen fixation. Hellriegel and Déherain had also found very large increase in the nitrogen content of sand in pots when exposed to light, but always accompanied by a development of algae. In the light of such results the conclusion has been drawn that the algae cannot assimilate free nitrogen alone but only in concurrence with soil bacteria, the former producing carbohydrates which are used by the bacteria as a source of energy for the actual nitrogen fixation. Winogradsky's organism showed a very low efficiency of fixation for sugar consumed and was also strictly anaerobic, so that, as fixation in soil had been observed to be most abundant under aerobic conditions and often considerable in amount, the probability seemed strong that other and more active bacteria remained to be discovered. In 1901, Beijerinck was able to report upon a couple of closely allied organisms which gave a very abundant growth under highly aerobic conditions in solutions of carbohydrates containing no combined nitrogen; these he named

Azotobacter chroococcum and *Azotobacter agilis.* The former was widely distributed in cultivated soils and even in dune sand, the latter was only found in river and canal waters. *A. chroococcum* was obtained from soil by the method of 'elective culture,' so successfully employed by Winogradsky for the isolation of the nitrifying organisms. A few grams of soil were introduced into a solution of the composition:

Tap water	100
Mannite	2
Dipotassium phosphate	·02;

and incubated at a temperature of from 20°–30° either in diffused daylight or in the dark. After a few days a strong film formed on the liquid, consisting mainly of the organism, and by transferring a little to an agar made with the same solution and allowing isolated colonies to develop, a pure culture was obtained.

The mannite can be replaced by dextrose, levulose, cane sugar, dextrin, propionates, butyrates, and acetates, all of which are oxidised, according to Beijerinck, to carbonic acid and water. In mannite solution the growth is both active and fairly pure, whereas in dextrose more foreign organisms develop and cause acidity; and in propionates, butyrates, &c., the growth is very pure but less rapid and abundant. Beijerinck secured a fixation of nitrogen up to 7 mgs. for every gram of mannite or dextrose oxidised in the cultures directly seeded with soil, and similar amounts by inoculating repeatedly with the impure film. In pure culture the *Azotobacter* refused to grow and fix nitrogen in two cases out of four and in the other two fixed 2·70 and 5·50 mgs. N per litre of culture, or only ·13 and ·27 mgs. N for 1 gram of carbohydrate. By combining pure cultures of it with spore-forming butyric organisms (*Granulobacter* sp.) or with non-spore formers separated from the films (*Radiobacter* and *Aerobacter*), he obtained better fixation, up to 5·9 mgs. N per gram sugar consumed. He made no attempt to get *Granulobacter* species alone to fix nitrogen and his attempts with *Radiobacter* and *Aerobacter* were also unsuccessful; nevertheless he asserts that all *Granulobacter* sp. can fix nitrogen and that *Azotobacter* cannot do so alone (although the above-mentioned results disprove the statement). Beijerinck supposes that in mixed cultures the *Granulobacters, Radiobacter* and *Aerobacter* gain the power to fix in the presence of *Azotobacter*, which grows itself at the expense of the combined nitrogen escaping from them into the solution.

Very soon, however, Gerlach and Vogel, Freudenreich and Koch, showed that *A. chroococcum* was able to fix considerable quantities of nitrogen in perfectly pure culture. Gerlach and Vogel found that old cultures of the *Azotobacterium* became weaker in their fixative power, and that the amount of N fixed per gram of sugar consumed depended both upon aeration and the concentration of the sugar; 12 grams per litre was found most favourable, giving a fixation of 9–10 mgs. N per gram of sugar oxidised.

Freudenreich has found about the same ratio for cultures on gypsum, and Koch for those on agar. Gerlach and Vogel have proved that the organism cannot grow without phosphoric acid and calcium, but can do without magnesium and potassium. As regards potassium their results are not conclusive, since while as much N was fixed in 20 days without it as in a culture to which it was added, after 40 days there had been no further fixation in the former solution, but it had nearly doubled in the latter, suggesting that the traces of potash in the chemicals and dissolved from the glass during sterilisation had been enough to carry on development for a time only.

The Author's Observations.

In order to gain the first or impure culture of *Azotobacter* from soil a solution of the following composition has been used:

Mannite	12 or 20 g.
Monopotassium phosphate ...	·2 g.
Magnesium sulphate cryst. ...	·2 g.
Sodium chloride	·2 g.
Calcium sulphate	·1 g.
Distilled water	1 litre.

The phosphate was dissolved separately and rendered just alkaline to phenol-phthalein by the addition of N/10 sodium hydrate. 75 c.c. or 100 c.c. of the solution was then put into Erlenmeyer flasks of 250 or 300 c.c. capacity and sterilised in an autoclave at 130° for 15 minutes. Each flask then received ·5 g. of calcium carbonate (precipitated) and 1 g. of soil. Incubation was maintained at about 30°. After two days the solution becomes somewhat clouded and gas bubbles rise from the bottom of the flask; on the third day a gelatinous colourless ring forms on the glass along the edge of the liquid and from it a film rapidly extends and covers the whole surface of the liquid; this is at first white, opaque, dry, often very reticulate, and it may or may not be filled with gas bubbles. After a couple of days

it becomes yellow, later brown and finally almost black. The last state is arrived at in from 7–10 days, and the liquid beneath is usually quite clear. By the tenth day the mannite has usually disappeared, sometimes even at the end of a week. If allowed to remain after the disappearance of the carbohydrate the solution begins to cloud again and the film breaks up and falls to the floor of the flask; at the same time a marked putrefactive odour is perceived, due to the attack on the proteid of dead *Azotobacter* cells by putrefactive bacteria. If it is proposed to determine the amount of nitrogen fixed this latter stage must not be allowed to proceed since it results in loss of nitrogen as ammonia and other volatile compounds. The nitrogen has been determined by transferring the whole contents of the flasks to Jena glass combustion flasks, acidulating with sulphuric acid and evaporating to a small volume before adding the concentrated acid. As a rule there is an odour of butyric acid and fruit ether from the culture, the acid odour being most marked during concentration. When the butyric odour is much in evidence *Clostridia* can be found in flocks overlying the carbonate and soil on the bottom of the flask but rarely in the surface film, and this is accompanied by a relatively poor fixation of nitrogen. This occurs more often in cultures to which no calcium carbonate has been added. To obtain pure cultures a little young film is well shaken in sterilised distilled water and a drop spread over mannite agar in a petri dish. The agar must first be well washed for some days with frequent changes of cold water after cutting up, then partly dried with the aid of the filter pump, and finally dissolved in the culture solution mentioned above in the proportion of $1\frac{1}{2}$–2 g. per litre. If unwashed agar is used too many colonies of foreign organisms develop before the *Azotobacter* and fuse with its colonies. Even in purified mannite agar transparent watery colonies of fluorescent bacteria appear in one day, and the *Azotobacter* colonies which appear about a day later often grow over them or fuse with them so that one cannot depend on getting a pure culture by inoculating off the first plate. In a second plating one can generally find colonies of *Azotobacter* isolated enough to secure a pure culture after transferring to an agar slant.

Appearance of Azotobacter chroococcum.

In the young impure films on solutions inoculated with Rothamsted soil the organism appears at first mainly in oval forms, often united in pairs, but also as cocci and diplococci, the former measuring 4–5 μ in length by 3 μ in width and the latter 2·5–4 μ in diameter for the

individual cell. If stained with iodine in potassium iodide many only assume a golden yellow colour, others are more brown and many of the cocci are of a deep red-brown. This latter colouration has been shown by Heinze to be due to glycogen similar to that which occurs in yeast cells. In old cultures the cells are practically all stained red-brown by iodine. If a little of a very young film is examined in a hanging drop of culture liquid, a cell here and there will be observed to suddenly become sluggishly motile and slowly cross the field of vision. Zettnow has found that the motion is due to a bundle of cilia at one pole. On mannite agar the colonies first appear as milky white glistening drops, round and convex, which under a low magnification show a coarsely granular structure extending to the margin. The colonies rapidly increase in size and after a week or more become brown at the centre, with concentric rings alternating dark and white to the circumference and darker streaks radiating from the centre outwards. In old cultures, where the agar has partly dried up, the cells are often united in Sarcina-like packets, the cell walls are much swollen, and the contents are aggregated to a small ball at the centre; at the same time giant cells both round and elongated can be seen filled with oil drops, and often a number of involution forms drawn out into long threads with false septa.

Some cultures after repeated transference on agar slants lose the power of turning dark with age and do not again recover it. On peptone beef extract agar the organism gives hardly any growth, but grows well when 1 per cent. dextrose is added, though the power of forming glycogen seems to be lost on this medium. In broth containing no added sugar there is practically no growth, the medium remaining clear with the formation of a slight sediment; if dextrose is added there is abundant multiplication at the surface of the liquid and a considerable sediment. To test the purity of cultures, seeding has been made into broth, and plates have been prepared with peptone beef extract agar with dextrose added; if the culture was pure the only colonies appearing on the peptone dextrose medium were those of *Azotobacter*.

Fixation of nitrogen by pure cultures.

In some early experiments with pure cultures of the Rothamsted organism great difficulty was found in getting it to grow in solutions containing either mannite or dextrose and prepared either with tap water or distilled water. After five weeks there was very slight

multiplication, and the quantity of nitrogen fixed was infinitesimal, although the cultures used had been freshly isolated, and were growing vigorously on the solid medium. Later, in order to secure an abundant inoculation the following method was adopted: 50 c.c. portions of dextrose and mannite culture solutions containing 12 g. to the litre and made up with tap water, received ·5 g. calcium carbonate, and were then sterilised. Flasks of 250 c.c. capacity received 10 c.c. of dextrose or mannite agar, and were then sterilised. The culture of *Azotobacter* was rubbed over the surface of this agar and allowed to develop for two or three days at 30°. When the growth had become abundant 50 c.c. of a sterile liquid medium, made up with tap water containing 12 g. of either mannite or dextrose in the litre and 1 per cent. calcium carbonate, was poured on to it and the flasks incubated at 30°. As a result of this method of inoculation the organism multiplied abundantly and formed a film on the surface of the liquid. Controls prepared in the same way but not inoculated were incubated with the cultures.

The results, with these pure cultures of organisms from three different places are given in the following table:

TABLE I.

Nitrogen fixed in milligrams for 1 gram of carbohydrate.

Origin	Carbon source	Time	1st plating	2nd plating	3rd plating
		days	mgs.	mgs.	mgs.
Mombasa, E. Africa	mannite	20	7·30	5·12	7·24, 7·82
	glucose	20	6·73	6·18	7·85
Cairo, Egypt	mannite	20	7·64	—	—
	glucose	20	5·73	—	—
Rothamsted	mannite	40	4·91	3·77	—
	glucose	40	4·62	3·57	—

A further reference will be made to the organisms from Africa under the final heading.

To test whether the mannite had disappeared, a drop or two of the solution was evaporated to dryness on a watch-glass and examined under the microscope for the characteristic needles; for dextrose by running a little of the culture into a boiling solution of methylene blue in caustic potash and observing whether the colour was discharged. Both tests were employed by Beijerinck.

Fixation was considerable in all cases and with the Mombasa *Azotobacter* was not weakened after three successive platings. The Rothamsted organism developed much more slowly, and gave a lower yield of nitrogen, thus showing a marked diminution in power to fix after the second plating..

Fixation of nitrogen by impure cultures.

Soil from many of the Rothamsted plots has been tested for the presence of *Azotobacter*, and the amount of nitrogen fixed in solutions seeded with it has been determined.

The following experiment brings out the influence of aeration on nitrogen fixation. The solution used contained 20 g. of mannite in the litre. The cultures were incubated at 30° for ten days, and each received 1 g. of soil as inoculation, and ·5 g. $CaCO_3$.

Soil	Solution	Total N fixed	N fixed per 1 g. mannite
Barnfield		mgs.	mgs.
1 c.	100 c.c.	11·9	5·95
,,	50 c.c.	9·2	9·20
4 c.	100 c.c.	9·1	4·55
,,	50 c.c.	8·8	8·80

The flasks used were of 250 c.c. capacity in which 50 c.c. of solution only forms a thin and fully aerated layer.

It is evident that the superior aeration with only 50 c.c. of solution caused a much greater fixation of nitrogen per gram of mannite oxidised. A stronger film of *Azotobacter* also developed.

In the following table will be seen the amounts of nitrogen fixed both where *Azotobacter* developed and where no trace of it could be found.

TABLE II.

50 c.c. solution, 2 g. soil and incubated 30 days at 25°–30°.

Soil	N fixed for 1 g. mannite	*Azotobacter*
	mgs.	
Broadbalk Wilderness + $CaCO_3$	8·80	abundant
,, ,, ,, alone......	7·30	,,
Soil from drain gauge + $CaCO_3$	6·60	fairly abundant
,, ,, ,, alone......	5·10	,,
Geescroft Wilderness + $CaCO_3$	3·53	absent
,, ,, ,, alone	2·60	,,
Harpenden Common + $CaCO_3$	3·73	,,
,, ,, ,, alone......	2·90	,,
Park soils		
Plot 1, unlimed........	3·94 and 1·15	,,
,, limed.........................	3·66 and 3·80	,,
Plot 4·2 unlimed	3·59	,,
,, limed	3·35	,,
Plot 9 unlimed	3·21	,,
Average fixation	6·95	*Azotobacter* present
,, ,, ...	3·22	,, absent

In presence of *Azotobacter* the average yield of nitrogen was more than doubled. When *Azotobacter* was absent there was a long continued production of gas and a foamy very thin film, a strong odour of butyric acid and flocks of *Clostridia* on the bottom of the flasks. It is evident then that fixation of nitrogen takes place even in the absence of *Azotobacter*, but is always very low in amount, approaching the yield found by Winogradsky for his *Clostridium*. It may be added that quite recently Thiele has obtained from soil several *Clostridia*, distinct from *C. pasteurianum* and capable of fixing up to 3 mgs. of nitrogen for every gram of dextrose fermented. Some of them also ferment mannite.

In all experiments with Rothamsted arable soils where fixation of nitrogen occurred *Azotobacter* was abundant in the film on the cultures.

The following are the best results obtained with pure and crude cultures of the organism.

Pure cultures	Mixed soil cultures
mgs. N fixed for 1 g. mannite oxidised	mgs. N fixed for 1 g. mannite oxidised
7·30	9·2
7·64	8·8
7·24	8·8
7·82	9·2
Average 7·50	9·22
	9·53
	Average 9·12

It seems that in some way concurrence with other bacteria in the primary cultures acts favourably on fixation, provided the reaction remains neutral or alkaline; free acid (as in cultures without calcium carbonate which give a butyric fermentation) seriously affects the growth of *Azotobacter*.

Conditions favourable for fixation of Nitrogen.

1. *Aeration.*

The value of an abundant supply of air is well shown in the results of Table I.; in this case a solution of only half the depth with a similar extent of surface gave nearly twice as much fixation per gram of mannite oxidised. The more rapid growth on solid media as against liquids is also a proof of the great requirements of the organism for oxygen. In shallow cultures the injurious butyric fermentation, which is more or less anaerobic, is partly or wholly suppressed.

2. *Presence of a base.*

In Table II. the amount of fixation in the presence and absence of calcium carbonate is shown. It will be seen that where *Azotobacter* was present the addition of base increased fixation. In the following experiment the amount of nitrogen gained was determined at the end of ten days in 100 c.c. solutions containing 2 g. of mannite, with and without the addition of ·5 g. calcium carbonate. The solutions were inoculated with 1 g. of soil and incubation was conducted at 29°–30°.

Soil	Depth	$CaCO_3$ added	No addition of $CaCO_3$
		Nitrogen fixed mgs.	Nitrogen fixed mgs.
Little Hoos	10 cms.	13·13	5·33
" "	20 cms.	9·78	4·79
" "	30 cms.	5·21	3·98
Agdell, unmanured ...	10 cms.	9·36	4·61
" full manured...	10 cms.	5·86	0·00

The figures show the total amount of fixation, the mannite not having been all oxidised in most cases. The *Azotobacter* has developed more rapidly in the series with calcium carbonate, which has secured growth and fixation in all cases, whereas both were wanting in one of the solutions receiving no carbonate.

In the following experiment an attempt was made to judge of the distribution of *Azotobacter* in several plots on Agdell Field at Rothamsted, where a four course rotation has been maintained with and without manures for over 50 years. One half of the plots had been left fallow every fourth year, and the other half seeded with beans or clover. The manures, all applied to the root crop, were on one-third of the land, phosphates and potash only; on another third, the same mineral manures and nitrogen; and on the remaining third, no manure. At some time before the experiments began the no manure, and about half the mineral plots, had been heavily chalked in the past, but the other half of the mineral plots, and the minerals with nitrogen plots, were unlimed, containing at present only a trace of carbonate. The soil was taken at a depth of 15 cms., and 1 g. was seeded into 75 c.c. solutions containing 12 g. mannite to the litre. To some solutions ·5 g. calcium carbonate was added, to others the same amount of magnesium carbonate, but most were left neutral. The flasks were incubated at 30° degrees for twelve days. Early in May, 1906, when the samples were taken, one half of each plot was under alsike clover, and the other half in fallow.

TABLE III.

Plot	N fixed for 1 g. mannite			Film appeared after		
	Neutral	With $CaCO_3$	With $MgCO_3$	days	days	days
	mgs.	mgs.	mgs.	Neutral	$CaCO_3$	$MgCO_3$
Minerals-fallow,						
chalked end	6·50	—	—	$3\frac{1}{2}$	—	—
unchalked end ...	4·75	6·80	8·00	4	4	6
Minerals-clover,						
chalked	5·80	—	—	5–6	—	—
unchalked	0·00	4·80	9·22	none	5	6
Unmanured-fallow,						
chalked	6·90	—	—	5–6	—	—
Unmanured-clover,						
chalked	4·30	—	—	7	—	—
Full manured-fallow,						
unchalked	4·20 & 0·00	5·80	9·53	4 & none	5	8
Full manured-clover						
unchalked	0·00	3·78	0·00	none	8	none

All the cultures were made in duplicate, and the figures represent the mean fixation for the pairs. The figures for the neutral solutions show that *Azotobacter* developed in every case from the fallow soils whether containing carbonate of lime or not; two of the clover soil inoculations failed to show any growth of *Azotobacter* and were not analysed, the average fixation from the fallow being 5·06, and from the clover land only 2·52. The chalked parts always gave *Azotobacter*, but there were three failures from the unchalked land, the average fixations being 5·87 and 1·71 respectively. Where there was failure to develop *Azotobacter* in the neutral solution, addition of calcium carbonate secured a film in each case. From the results with the neutral solutions it may be concluded that *Azotobacter* is more abundant in fallow than under clover, and much more abundant in soils well provided with carbonate of lime than in others where the latter is almost absent. The results shown in Table II. present a striking confirmation of the latter conclusion. In the three cases where *Azotobacter* developed and nitrogen fixation was large the soils contained abundance of calcium carbonate. In every case where *Azotobacter* failed to appear, the soils contained only a trace of carbonate, and even addition of carbonate to the solutions was ineffectual. All the latter soils were acid to litmus.

Magnesium carbonate as base.

As shown in Table III., an addition of magnesium carbonate in place of calcium carbonate caused a very large fixation of nitrogen in three cultures out of four, but the film developed much later. The average fixation with magnesium carbonate was 8·92 mgs., with calcium carbonate 5·80 mgs. The film developed with magnesium carbonate in 6·6 days and with calcium carbonate in 4·6 days. With magnesium carbonate there was 50 per cent. more nitrogen fixed, and a delay of two days in development. Examination of the cultures showed that the film with magnesium carbonate contained far less foreign organisms, and that whereas with calcium carbonate there was a butyric or fruit ether odour, the cultures with magnesium carbonate were quite odourless, even during concentration with acid.

A special experiment was therefore made to compare the influence of the carbonates of magnesium and calcium upon fixation and growth.

To three parallel solutions of 75 c.c. containing 12 g. mannite to the litre, ·5 g. calcium carbonate was given, to another set ·5 g. magnesium carbonate, to a third ·25 g. of each carbonate, and another was left neutral. All were inoculated with ·5 g. soil and incubated for 14 days

at 29°–30°. The amount of nitrogen fixed is expressed as mgs. per gram mannite.

	Neutrals	$CaCO_3$ series	$MgCO_3$ series	$CaCO_3+MgCO_3$
1.	5·75	7·8	8·12	8·71
2.	4·98	7·15	7·62	7·08
3.	—	6·70	8·25	—
Average	5·36	7·22	8·00	7·89
Film appeared in days	4–5	3–4	8–10	6

During concentration the neutral cultures developed a strongly acid odour, those with calcium carbonate a weaker one, and those with magnesium carbonate, alone or mixed with calcium carbonate, gave no odour. When magnesium carbonate was present, development was greatly delayed, but the yield of nitrogen was again larger, though not to so marked an extent as in the earlier experiment. In pure culture *Azotobacter* gives rise to no acidity, either in solutions or on agar. One must conclude, therefore, that magnesium carbonate not only neutralises more effectually than calcium carbonate any trace of acidity due to foreign organisms in the early stages of culture, but also prevents butyric fermentation, but at first it inhibits the growth of *Azotobacter* itself.

Nitrogen fixation by soils taken at different depths.

Only one experiment has been made in this connexion. The writer has described in another place a method for obtaining and preparing an average soil sample[1]. The soil was taken from a fallow plot at depths of 10, 20 and 30 cms., and equal quantities were seeded into 100 c.c. portions of a culture solution containing 20 g. mannite in the litre. ·5 g. calcium carbonate was added, and incubation maintained at 29°–30°.

Little Hoos fallow soil.

Depth	7 days	10 days	13 days
	mgs.	mgs.	mgs.
10 cms.	6·84	13·13	12·15
20 cms.	3·77	9·78	11·03
30 cms.	2·93	5·21	10·20

[1] "The comparative nitrifying power of soils," *Trans. Chem. Soc.* 1904, vol. LXXXV., p. 1158.

The figures show the total nitrogen gained by the cultures during the times stated. The greatest contrast was after ten days. During this time the mannite had all been oxidised in the cultures inoculated with soil at 10 cms. depth, so that no further gain of nitrogen took place during the remaining three days. With soil from 20 cms. depth most of the sugar had gone after ten days, but with that from 30 cms. not till after thirteen days. The results show that *Azotobacter* is most abundantly present in soil near the surface, and falls off in amount with increasing depth. It is also evident that fixation does not occur uniformly over the period of active oxidation, but increases rapidly up to a point, and then becomes slower, corresponding with the greatly lessened concentration of carbohydrate.

Azotobacter from African soils.

It had been observed that, when a little of a soil which gave a growth of the organism in solutions was rubbed over a mannite agar plate, the characteristic colonies develop in two or three days, together with many other forms which, however, soon cease to grow, while the *Azotobacter* colonies spread rapidly and soon begin to darken.

Mr Hall took with him to South Africa in 1905 a number of such plates, and inoculated them with fresh soil from several parts of the "high veldt," and one with a tropical cultivated soil at Mombasa, in E. Africa. On examination it was found that *Azotobacter* had developed in only two cases, giving a gelatinous nearly black growth, which covered the greater part of the plates. An attempt was made to get the organism in pure culture by growing in nutritive solutions. The material was very impure and contained many amoebae and infusoria, which live largely on the cells of *Azotobacter*. The Mombasa organism was obtained pure by plate inoculations from a film which had slowly formed on a solution of sodium butyrate. The other organism, from a "vlei" soil near Lichtenburg in the Transvaal, did not give even a relatively pure growth, and could not be isolated. The film of the Mombasa organism on the butyrate was white, dry and brittle, and was remarkable for the very large size of the round cells, 5–6 μ diameter, which were rarely united. In pure culture, this Mombasa organism differs from all others examined by the production of a soluble pigment, at first greenish-blue, and later, light yellow in tint, which not only colours the growth on agar, but diffuses into both the solid and liquid media. From the first, the pure culture had no power of turning brown with age. On mannite and

glucose agar the growth is very rapid and more fluid than that given by Rothamsted organisms, and under the microscope the cells are isolated, very small, and almost invariably round; the diameter varying from 2–2½ μ. Iodine in potassium iodide only stains it yellow, so that the power of forming glycogen seems to have been lost. The formation of the pigment is markedly favoured by the presence of calcium carbonate both in solid and liquid media. On one mannite agar plate, where carbonate had been first added but had accumulated at one end, the growth above the carbonate alone produced pigment, which was quite absent on the portion where carbonate was wanting. Calcium carbonate seems to have a specific action on the production of pigment, the colour of which is at once discharged by mineral acids.

The very active fixation of nitrogen by pure cultures of the Mombasa organism even after repeated transference, has already been referred to (see Table I.). Its behaviour towards media containing organic nitrogen is similar to that described for the Rothamsted organism. In a solution containing dextrose and ·02 per cent. nitrogen as calcium nitrate growth is abundant, and the nitrate is very slowly and only partly converted to ammonia.

The Cairo organism was isolated from a sample of fresh soil collected at Ghezirah by Mr R. Aladjem of the Agricultural Experiment Station there. This soil showed a markedly alkaline reaction to litmus paper, due to alkaline carbonate, but the bulk of the carbonate present was calcium carbonate. The crude cultures inoculated with soil were remarkable for the speed with which the film developed and the mannite disappeared, fixation being complete in five days, by which time the film was quite black. The fixation was also identical in amount from cultures with varying amounts of solution, and with and without added carbonate. Even after ten days there was no putrefactive odour, and no trace of a butyric fermentation could be detected. The fixation was in every case 9·2 mgs. nitrogen for 1 gram mannite oxidised.

In pure culture this organism is in every way similar to that from Rothamsted, with the single exception that fixation is with it more rapid and greater (see Table I.) in amount. The power of turning brown, and finally black with age, has been preserved in the pure cultures.

Although the vlei organism from S. Africa was not isolated, it produced a pigment quite similar to the Mombasa form in impure culture, and must certainly be classed with it as one variety. As descriptions by continental authors agree fully with the Rothamsted and Cairo type, one must conclude that there is one variety common to

Europe and extending to Egypt, and another quite distinct found in East and South Africa.

The organism separated by Beijerinck from waters and named *A. agilis*, is a larger form which is very actively motile in young liquid cultures, and also forms a diffusible pigment, green in solutions of salts of organic acids and reddish-violet in the presence of carbohydrates.

Effect of desiccation on Azotobacter chroococcum.

No spores are formed, yet the organism can resist drying up in the air for a long time. A soil, which was known to contain the organism in the fresh condition, after being kept air-dry in bottle for a year, still yielded an abundant growth after inoculation into a mannite solution. Old cultures of the organism on agar which had dried down to a leathery consistency, after many months still showed abundant growth after pouring a fresh culture solution over them. It is evident then, that the organism can be freely distributed in dust by the wind.

General Remarks.

Several observers have been struck by the resemblance of the organism to some of the unicellular Algae. Beijerinck believes it to be closely related to Winogradsky's *Chromatium*, while Benecke and Keutner consider it a colourless form of one of the *Cyanophyceae* namely *Aphanocapsa*, but no one has yet been able to induce it to produce chlorophyll by cultivation in light. Attempts have been made to bring about nitrogen fixation by seeding pure cultures into sterilised and unsterilised soil, but as yet without success. Similar experiments are now in progress at Rothamsted. A. D. Hall has recently reported on two cases of considerable nitrogen increase in Rothamsted soils allowed to run wild for many years. In one, showing the greater increase, *Azotobacter* was abundantly present, in the other it could not be found, but butyric organisms were present (see Table II.).

Some references to the more important papers dealing with the subject are given below. Where the *Centralblatt für Bakteriologie* is cited the Second Part is always meant.

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SOME OBSERVATIONS ON "NITRIFICATION."

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It is now a well-established fact that the ammonia, which is formed in the cultivated soil by the breaking down of nitrogenous organic matter through the action of bacteria and moulds, is itself converted by oxidation into nitrates. The latter process, "nitrification" proper, is effected in two stages by two distinct species of bacteria, the one carrying the oxidation to nitrite, and the other changing the latter compound into nitrate. It is also known that, in the absence of a base, nitrification does not take place. The most usual and most available soil base is calcium carbonate, which is, however, present in some arable soils to only a very small amount, less than ·05 per cent.; in such soils, and in others where its presence cannot be detected, nitrification is still active, a fact which seems to suggest that other substances may replace it and serve as bases for nitrification.

In order to test the latter point an attempt has been made to set up nitrification in a solution containing an ammonium salt the necessary base being supplied by such commonly occurring soil-constituents as kaolin, modelling clay, and ferric hydrate, the latter both in the freshly precipitated form and as "iron rust." The solution had always shown itself very favourable for nitrification in the presence of calcium carbonate; the composition was as follows:

Ammonium sulphate	·5 gram.
Sodium chloride	·5 "
Cryst. magnesium sulphate	·15 "
Cryst. ferrous sulphate	·10 "
Monopotassium phosphate	·25 "
Distilled water	1 litre.

In a first experiment 100 c.c. portions were sterilised in Erlenmeyer flasks of 250 c.c. capacity, and one gram of either kaolin, modelling clay or freshly precipitated ferric hydrate was added, together with ·2 gram of a soil possessing active nitrifying power in order to convey the necessary organisms. After sterilisation the solution had an acidity equal to 2·3 c.c. N/10 alkali, with phenol-phthalein as indicator. For each substance there was a pair of acid and a pair of neutral solutions. Incubation lasted for 49 days at 29°–30° C. At the end of that time the cultures were examined for ammonia, nitrite and nitrate. In both the acid and neutral solutions containing kaolin and modelling clay the ammonia reaction was strong, that for nitrite absent and the nitrate reaction very faint; with ferric hydrate there was still ammonia but also a strong nitrite reaction. A pair of control solutions to which ·2 calcium carbonate had been added nitrified completely in 20 days. The total absence of nitrification in the flasks containing kaolin and modelling clay after seven weeks (the trace of nitrate was from the air) indicates that those substances cannot act as bases for nitrification. To try and confirm the result for ferric hydrate, a further series of cultures were made both with the freshly precipitated form and with finely ground "iron rust." The quantitative results are set out in Table I.

TABLE I.

Base	Period of incubation	Reaction	N as Ammonia	N as Nitrite	N as Nitrate	Total N	Parts p.c. nitrified	Excess of nitrified N due to base
1st Set	days		mgs.	mgs.	mgs.	mgs.		
Ferric hydrate 1.	49	acid	7·13	2·95	none	10·08	29·3	
" " 2.	"	"	7·35	2·45	"	9·80	24·9	
" " 1.	"	neutral	6·44	3·20	"	9·64	33·2	
" " 2.	"	"	6·30	3·45	"	9·75	35·4	
2nd Set								
Ferric hydrate 1.	32	acid	7·42	none	2·80	10·22	27·4	20·2
" " 2.	32	"	7·39	"	2·70	10·09	26·7	19·5
" " 3.	43	"	7·21	"	3·30	10·51	31·4	24·2
" " 1.	43	neutral	5·60	4·34	none	9·94	43·6	17·9
" " 2.	43	"	5·81	4·10	"	9·91	41·4	15·7
Iron rust 1.	32	acid	6·30	none	4·06	10·36	39·2	32·0
" " 2.	32	"	6·30	"	4·06	10·36	39·2	32·0
" " 3.	43	"	6·23	"	4·13	10·36	39·9	32·7
" " 1.	43	neutral	4·62	"	5·67	10·29	55·1	29·4
" " 2.	43	"	4·20	"	5·98	10·18	58·7	33·0
Soil alone	43	acid	9·94	"	0·77	10·71	7·2	
Soil alone 1.	43	neutral	7·77	"	2·80	10·57	26·5	
" " 2.	43	"	7·77	"	2·60	10·43	24·9	

Excluding the first set, in which no controls with soil alone were made, it will be observed that, after allowing for the nitrification in the controls of the second series, the ferric hydrate has accounted for the oxidation of about 20 per cent. of the ammoniacal nitrogen and the "iron rust" for over 30 per cent., and that there is practically no increase after 32 days. Nitrification has therefore stopped at a certain point in spite of the great excess of base. This result is most probably due to the hydrolysis of the ferric nitrite or nitrate formed, the acid being set free and preventing further action when it had attained a certain concentration. Soil conditions would be more favourable for the continuation of nitrification, especially below vegetation actively withdrawing the nitric radical. One may conclude therefore that ferric hydrate may act as a soil base inferior to calcium carbonate.

Nitrification of Ammonia absorbed by Clay and Peat.

It is well known that both clay and peat absorb the ammonia of an ammonium salt, the action being a substitution whereby the ammonia displaces a base from its combination with hydrated aluminium silicate in the one case and with humic acid in the other; the base comes into solution combined with the acid of the ammonium salt, so that no change in the reaction of the medium can be observed. With regard to clay this only holds true if the solution of the neutral salt is weak, as Veitch has shown that 5 per cent. solutions of neutral salts of the alkalis give rise to aluminium and ferric salts which hydrolyse and produce an acid solution.

It was thought of interest to observe whether the double ammonium salts formed with peat and clay were susceptible of nitrification in the absence of any specially added base. It may be added that these combinations do not yield any ammonia to pure water.

For the experiment modelling clay and a close black homogeneous peat taken from some depth were used. 100 grams of modelling clay were shaken for twelve hours with 3 litres of N/50 ammonium chloride solution, and 100 grams of peat were similarly treated with 2 litres of the same solution. 5 grams of the washed materials were added to 100 c.c. portions of a nitrogen-free mineral solution, together with ·2 gram of a soil having active nitrifying power. Control solutions contained ·1 gram calcium carbonate in addition to either clay or peat. The medium had the following composition:

Cryst. magnesium sulphate	...	...	...	...	50 mgs.
Sodium chloride	...	...	...	...	50 „
Monopotassium phosphate	...	...	...	...	50 „
Distilled water	...	...	...	...	1 litre.

The solutions were either left acid or made neutral to phenolphthalein. Incubation lasted 60 days at 30° C. The quantitative results are given in Table II.:

TABLE II.

Substance	Reaction	N as Ammonia	N as Nitrate	Total Nitrogen
Modelling clay ....1.	acid	0·42	1·96	2·38
„ „ ....2.	„	0·28	1·96	2·24
„ „ ....1.	neutral	0·84	—	—
„ „ ....2.	„	0·56	2·10	2·66
„ „ + ·1 g. $CaCO_3$ 1.	„	0·14	2·66	2·80
„ „ „ 2.	„	0·21	2·24	2·65
Peat ....1.	acid	1·68	0·56	2·24
„ ....2.	„	1·82	0·28	2·10
„ ....1.	neutral	1·82	0·42	2·24
„ ....2.	„	1·82	0·42	2·24
Peat + ·1 g. $CaCO_3$ ....1.	„	0·14	2·10	2·34
„ „ ....2.	„	0·00	2·24	2·24

No nitrite was found in any case. It will be observed that both with the clay and the peat an addition of calcium carbonate has caused a practically complete nitrification of the absorbed ammonia. The modelling clay cultures without base show also a nearly complete nitrification, but this is by no means the case with the peat, the small amount of nitrate found in the absence of base having come from the air. The ammonia absorbed by peat appears not to be nitrifiable in the absence of base, but is so when with clay. The conclusion would have been, however, more convincing with larger quantities of material.

IS A NEUTRAL AMMONIUM SALT DIRECTLY NITRIFIABLE?

In agricultural text-books the statement is often found that nitrification does not progress except in the presence of a base which neutralises the acid formed. The implication is that at the outset some nitrous acid is formed which, unless neutralised by a base, inhibits

further oxidation of ammonia by the organism. This occurrence of free acid has, however, never been proved. Winogradsky observed that perfectly neutral solutions containing ammonium sulphate inoculated with pure cultures of the nitrite organism showed neither at first nor after long keeping any trace of nitrification. Warington has also shown that a dilute ammonium carbonate solution (or a diluted stale urine) seeded with the organism nitrifies to the extent of exactly half the ammoniacal nitrogen, ammonium nitrite being produced, or, in the presence of both organisms, ammonium nitrate. Neither the sulphate nor the nitrate of ammonium are capable therefore of any degree of nitrification in the absence of a base. Both observers have concluded that ammonium carbonate is alone nitrifiable, but only completely so if another carbonate is present to decompose the nitrate of ammonium produced and form fresh ammonium carbonate. In the course of work upon the comparative nitrifying power of soils the writer has observed that control culture solutions to which insoluble carbonates have been added, but no organisms, show after some weeks at constant temperature a marked loss of ammoniacal nitrogen. This loss is due to a reaction between the ammonium sulphate in solution and the added carbonate, ammonium carbonate and a sulphate being formed, the former gradually volatilising from the solution. The amount of loss of ammonia depends upon the rate of formation of ammonium carbonate, which, other conditions remaining constant, depends upon the amount and the kind of carbonate added. The solution from which these losses have been observed had the following composition:

Ammonium sulphate	·5 gram.
Sodium chloride	·5 "
Monopotassium phosphate	·25 "
Cryst. magnesium sulphate	·15 "
Cryst. ferrous sulphate	·10 "
Distilled water	1 litre.

100 c.c. portions were sterilised in Erlenmeyer flasks of 250 c.c. capacity in a steam autoclave at a temperature of 125° for 15 minutes. During sterilisation no loss of ammonia occurred, nor was there any change in the ammonia content after incubation at 30° for two months. During sterilisation the iron was changed to the ferric state and precipitated as phosphate, the solution showed an acidity equal to 2·3 c.c. N/10 alkali with phenol-phthalein as indicator. The solution contained about 10·5 mgs. ammoniacal nitrogen. ·2 gram calcium carbonate, ·17 gram

magnesium carbonate and ·25 gram copper carbonate in a finely precipitated state were added to the unneutralised solutions and incubation maintained at 30° for varying periods; the losses of ammoniacal nitrogen with the different carbonates are shown in the following table:

	$CaCO_3$	$MgCO_3$	$CuCO_3$
Loss in 20 days			
1.	0·87 mgs.	2·3 mgs.	—
2.	0·70 ,,	2·1 ,,	—
3.	0·85 ,,	2·2 ,,	—
4.	0·98 ,,	2·5 ,,	—
Average loss	0·85 (8·02 p. c.)	2·27 (21·41 p.c.)	—
Loss in 30 days			
1.	1·18	—	—
2.	0·98	—	—
3.	1·16	—	—
4.	1·01	—	—
Average loss	1·08 (10·19 p.c.)	—	—
Loss in 56 days	—	—	0·21 mgs. (1·98 p.c.)

It is evident that magnesium carbonate reacts much more rapidly with the ammonium salt in solution than calcium carbonate, causing a loss of ammonium carbonate by volatilisation nearly three times as great in the same time. This property must be connected with the often observed fact that magnesium carbonate sets nitrification going in a solution several days earlier than calcium carbonate. On the other hand, the very insoluble copper carbonate has reacted so feebly with the ammonium salt that even after eight weeks the loss of ammonia is below 2 per cent. A pair of solutions containing ·25 gram copper carbonate and seeded with ·2 gram of a soil having very active nitrifying power failed to show any trace of nitrification after 40 days at 30°, although control cultures with calcium carbonate had completely nitrified in 18 days. That the copper carbonate had exercised no poisonous action on the organisms was proved by the fact that when a little calcium carbonate was added at the end of the 40 days, the ammonia became completely nitrified in less than three weeks. The incapacity of copper carbonate to set nitrification going must therefore be correlated with its very low power of reaction with the ammonium salt.

Comparison of the figures for calcium carbonate after 20 and 30 days shows that the loss of ammonia is greatest during the early period, nearly

79 per cent. of the loss during 30 days having occurred during the first 20 instead of only 67 per cent. had the loss been uniform.

The above observations confirm the conclusions of Winogradsky and Warington, that an ammonium salt is not directly nitrifiable and that the function of the base in nitrification is to secure the formation of ammonium carbonate, which alone is directly nitrifiable. From this standpoint kaolin and modelling clay cannot set up nitrification because they cannot free ammonia from a neutral salt, and ferric hydrate acts positively because it does react with the neutral salt.

Influence of Ammonium Salts and Asparagin upon the Oxidation of Nitrites to Nitrates by Nitrobacter.

I. *Influence of Ammonium Salts.*

Winogradsky[1] was the first to observe that ammonium salts in very low concentration exerted a marked inhibiting action on the oxidation of nitrites to nitrates by Nitrobacter in mineral solutions. He found that when a little of a pure culture of the organism was seeded into a nitrite solution containing only ·0008 per cent. nitrogen as ammonium sulphate, the oxidation required twice as long to complete as in the nitrite solution alone. In the presence of ·015 per cent. ammoniacal nitrogen no oxidation of nitrite occurred even after months. He concluded therefore that ammonium salts behave as poisons towards the organism. Boulanger and Massol[2] were able to confirm these observations, but also found that when an ammonium salt was added to a solution in which the organism had already oxidised nitrite to nitrate, much higher concentrations were required to inhibit the action; ·009 per cent. ammoniacal nitrogen had no effect on oxidation, and even ·075 per cent. only postponed the completion of oxidation a few days. They therefore concluded that ammonium salts prevent the multiplication of the organism, but exercise very little influence on the actual oxidation when Nitrobacter has already abundantly multiplied.

The writer had already started some experiments along similar lines before the results of the two latter observers came to his notice. His object was to study the influence of ammonium salts upon the oxidation of nitrites in the presence of large inoculations of a fresh and active organism, and to observe whether an organism which had been already

[1] Article on Nitrification in Lafar's *Handbuch der technischen Mykologie*, 2nd edition.
[2] *Ann. Inst. Pasteur*, 17 July 1903, 18 March 1904.

accustomed to an ammonium salt could set up oxidation in a fresh nitrite solution containing ammonia.

Experiment I. This was arranged to show what effect the addition of an ammonium salt at the outset together with large inoculations of an organism, which had been previously grown in the entire absence of ammonia, would have on the oxidation of nitrite to nitrate. The culture solution used had the following composition, after Omeliansky:

Sodium nitrite	1·50 gram.
Ignited sodium carbonate	1·00 "
Monopotassium phosphate	0·50 "
Sodium chloride	0·50 "
Cryst. ferrous sulphate	0·40 "
Cryst. magnesium sulphate	0·30 "
Distilled water	1 litre.

30 c.c. portions of the above were sterilised in Erlenmeyer flasks of 125 c.c. capacity and divided into two sets, one receiving in addition 1 c.c. doses of a 3 per cent. ammonium sulphate solution. At this point there was present in each flask 9 mgs. of nitrite nitrogen, and in the ammonia series 6 mgs. of ammoniacal nitrogen also. The organism used for inoculation was taken from a culture in a nitrite solution of similar composition to the above, in which it had oxidised 120 mgs. of nitrite nitrogen to nitrate per 100 c.c. solution, the nitrite having been added in successive doses of 20 mgs. as the reaction for it disappeared. This organism had been at no period of its culture from the original arable soil in contact with an ammonium salt. The mixed liquid and sediment of this culture were well shaken and varying quantities inoculated into the above-mentioned solutions; three flasks containing nitrite and ammonium sulphate received 2, 2, and 5 c.c. inoculations, and four others containing only nitrite 1, 2, 4, and 5 c.c. inoculations. The cultures were incubated at a temperature of 30°. As the nitrite disappeared by oxidation in each series a fresh dose was added in the form of 1 c.c. of a 5 per cent. sodium nitrite solution; fresh additions of ammonia were also made to the ammonia series, in the form of 1 c.c. of a 3 per cent. ammonium sulphate solution. As the nitrite was oxidised in the latter set the ammoniacal nitrogen was determined by withdrawing 2 c.c. of liquid and Nesslerising, calculating the quantity of nitrogen as ammonia for the whole volume of liquid, which was measured on each occasion. The results of this experiment are given in Table III.

TABLE III.

Period of incubation	Controls Nitrite no Ammonia	Ammonium Sulphate series		
		2 c.c. inoculation *a*.	2 c.c. *b*.	5 c.c.
6 days	no NO_2 reaction	wk. NO_2 reaction	wk. NO_2 reaction	wkr. NO_2 reaction
6 days later	,,	no NO_2 reaction	no NO_2 reaction (3·9 mgs. N as NH_3 present)	no NO_2 reaction
7 days later	,,	no NO_2 reaction (7·60 mgs. N as NH_3 present)	—	wk. NO_2 reaction
6 days later	,,	no NO_2 reaction	—	no NO_2 reaction
7 days later	,,	no NO_2 reaction (11·0 mgs. N as NH_3 present)	—	wk. NO_2 reaction
9 days later	—	—	—	no NO_2 reaction (10·5 mgs. N as NH_3 present)
6 days later	no NO_2 reaction	no NO_2 reaction (26·2 mgs. N as NH_3)[1]	—	—
12 days later 59 days in all	,,	—	-	no NO_2 reaction (16·5 mgs. N as NH_3)[2]

[1] =0·075 per cent. N as NH_3 in the solution. [2] =0·050 per cent. etc.

It is evident that at first there has been an inhibition of oxidation in the presence of ammonium sulphate, twelve days being required to complete it as against only six in the controls. When inoculation had been made with 5 c.c. of the culture oxidation was more rapid, as shown by the weaker nitrite reaction after six days. A determination of the residual ammonia in one flask at the end of the twelve days showed that there had been considerable volatilisation from the alkaline liquid, but there still remained ammoniacal nitrogen equal to ·013 per cent., but little short of the quantity stated by Winogradsky (·015 per cent.) to completely stop oxidation. In the third period after the addition of fresh nitrite and ammonium sulphate, oxidation was complete in all the series, though the ammoniacal solutions now contained ·025 per cent.

ammoniacal nitrogen. By successive additions of nitrite and ammonium sulphate, oxidation could be made to proceed as rapidly in the presence of ·075 per cent. ammoniacal nitrogen as in the controls containing only nitrite. One must conclude therefore that with a sufficiently heavy inoculation at the outset much of the inhibiting action of an ammonium salt can be avoided, although the organism had had no previous opportunity of habituating itself to ammonia; the inhibition is however still perceptible in the presence of ·013 per cent. ammoniacal nitrogen. The subsequent rapid oxidation of nitrite, in spite of increasing quantities of ammonia, confirms the results of Boulanger and Massol.

Experiment II. The object here was to observe the effect of adding ammonium sulphate to solutions in which oxidation of nitrite had occurred already several times. For this purpose two of the control solutions from the previous experiment, which had converted three successive doses of nitrite to nitrate, received an addition of fresh nitrite, together with 8 mgs. of ammoniacal nitrogen as ammonium sulphate. These two solutions had originally received 4 c.c. and 1 c.c. inoculations of the organism. A control solution without ammonia was also incubated. The result was as follows:

Date	4 c.c. culture and Ammonia	1 c.c. culture and Ammonia	Control Nitrite only
6 days	no NO_2 reaction (6 mgs. N as NH_3 present = ·02 p.c.)[1]	wk. NO_2 reaction	no NO_2 reaction
2 days later	—	no NO_2 reaction	—
4 days later	no NO_2 reaction (·038 p.c. N as NH_3 present)[2]	—	no NO_2 reaction
4 days later 16 days in all	—	no NO_2 reaction (·047 N as NH_3 present)[3]	—

[1] = 0·02 per cent. N as NH_3 in solution.
[2] = 0·038 etc. [3] = 0·047 etc.

In the first period, with 4 c.c. of the inoculating medium, nitrification was as rapid in the presence of ammonium sulphate as in the control, although there was half as much again ammoniacal nitrogen present as at the end of the first period in Experiment I. It is here evident

that, where the organism has already strongly multiplied, an addition of ammoniacal nitrogen in excess of that stated by Winogradsky to prevent all oxidation has had no inhibiting effect.

Experiment III. The object here was to observe whether ammonia salts inhibit oxidation by an organism which had previously been habituated to high concentrations of ammonium salts. The organism employed was the one cultivated in the ammonia series of Experiments I. and II. The cultures were all united, well shaken, and in every case only 1 c.c. used for inoculation. Six sterile solutions of nitrite were prepared as previously described and treated as follows:

1, 2, and 3 received 1 c.c. inoculation and 10 mgs. ammonium sulphate.
4 „ only inoculation.
5 „ only ammonium sulphate.
6 „ no addition.

For the actual experiment only 1, 2, 3 and 4 are of interest. The flasks were incubated as before at 30°, and the ammonium sulphate and nitrite were renewed as in Experiment I., the ammonia present from time to time being determined in the manner there described. The results are shown in Table IV.

In order to bring out the significance of the results it is necessary to compare them with those obtained in Experiment I. There the presence of ·013 per cent. ammoniacal nitrogen doubled the time required for controls, here there has been no inhibition even in the presence of double the quantity of ammoniacal nitrogen, namely ·026 per cent., and with a weaker inoculation of the organism.

It is then evident that the nitrobacter, by previous cultivation in solutions with gradually increasing ammonia content, has acquired the property of withstanding the deleterious effect of the latter upon its growth.

In this series oxidation in the presence of as much as ·119 per cent. ammoniacal nitrogen has kept pace with the controls. Oxidation was finally stopped in both series alike, not by the ammonium sulphate concentration, but by the accumulation of the nitrate produced. A determination of the nitric nitrogen in the latter showed an amount equal to ·95 per cent. sodium nitrate.

TABLE IV.

Period of incubation	Culture 1. with Ammonia	Culture 2. with Ammonia	Culture 3. with Ammonia	Culture 4. (Control) Nitrite only
Dec. 24–Jan. 2 9 days	no NO_2 reaction (7·8 mgs. N as NH_3)[1]	no NO_2 reaction	no NO_2 reaction	no NO_2 reaction
Jan. 2–8 6 days later	—	no NO_2 reaction (15·8 mgs. N as NH_3)	wk. NO_2 reaction	,,
Jan. 2–11 3 days later	—	—	no NO_2 reaction (15·3 mgs. N as NH_3)	—
Jan. 8–13 2 days later	—	no NO_2 reaction (23·1 mgs. N as NH_3)	—	no NO_2 reaction
Jan. 11–16 3 days later	—	—	no NO_2 reaction (28·5 mgs. N as NH_3)	—
Jan. 13–19 3 days later	—	no NO_2 reaction (33·3 mgs. N as NH_3)[2]	—	no NO_2 reaction
Jan. 19–31 12 days later 38 days in all	—	str. NO_2 reaction (40·5 mgs. N as NH_3)	str. NO_2 reaction (35·0 mgs. N as NH_3)	str. NO_2 reaction

[1] = 0·026 p.c. N as NH_3 in the solution. [2] = 0·119 etc.

Loss of Nitrogen from the Nitrite Culture Solution containing Ammonium Sulphate.

It was thought that there might be a reaction between the nitrite and the ammonium salt leading to a loss from the solution in the form of nitrogen gas. To determine if this were so the solutions 5 and 6 were incubated with the cultures of Experiment III., and the ammonia and nitrite determined in 5 and nitrite in 6 after nine days. The ammonia was also determined in culture 1. The results were as follows:

Number	NH_3N	NO_2N
1.	7·80 mgs.	—
5.	8·0 ,,	8·80 mgs.
6.	—	8·90 ,,

There was evidently no loss of nitrogen by reaction between the nitrite and ammonia in flask 5. There was originally 10 mgs. ammoniacal nitrogen present, so that the only loss was due to volatilisation of ammonium carbonate from the alkaline liquid, amounting to 20–22 per cent. of the quantity added. That the loss was of this nature was shown by a determination of the alkalinity of 1, 5, and 6, cochineal being used as indicator.

To neutralise	1	required	3·3 c.c.	N/10 acid.
"	5	"	3·4	"
"	6	"	5·8	"

The reduction of alkalinity in 1 and 5 indicates loss of ammonium carbonate from the solution. The maximum amount of ammonium sulphate nitrogen converted to carbonate by the sodium carbonate present would be 8·30 mgs., or 83 per cent. of the quantity at first added. It may be assumed therefore that in the solution used for the three experiments a part of the ammonia, up to ·027 per cent., must have been in the form of carbonate, but no experiments have been made to decide the point as to whether the carbonate and sulphate of ammonium affect the growth and oxidising power of nitrobacter in different degree.

II. *Influence of Asparagin.*

Winogradsky has stated that asparagin in a concentration of ·70 per cent. prevents the oxidation of nitrite to nitrate.

Some experiments have therefore been made in the same manner as with ammonia, to determine whether by strong inoculation of the organism the inhibiting action of asparagin can be lessened or removed.

Experiment I. The object here was to determine whether a strong inoculation of an organism which had never been in contact with asparagin would bring about oxidation of nitrite in the presence of the above-mentioned asparagin concentration, namely ·70 per cent. of the solution.

The procedure was similar to that adopted for Experiment I. of the ammonia series, the organism used being the same also. The results are shown in Table V. A marked slowing of oxidation is evident at the outset, the controls having oxidised three successive nitrite doses before oxidation was completed in the asparagin series. The first oxidation required 19 days, but after a fresh addition of nitrite, oxidation was as rapid as in the controls. A determination of the asparagin in the culture inoculated with 5 c.c. of organism showed that at the end of

TABLE V.

Period of incubation	Controls Nitrite only	Asparagin ·72 p.c. of the solution		
		1 c.c. inoc. *a*.	1 c.c. inoc. *b*.	5 c.c. inoc.
6 days	no NO_2 reaction	str. NO_2 reaction	str. NO_2 reaction	mod. NO_2 react.
6 days later	,,	mod. NO_2 react.	mod. NO_2 react.	wk. NO_2 reaction
7 days later	,,	no NO_2 reaction (added NO_2 sn.)	no NO_2 reaction (added NO_2 sn.)	no NO_2 reaction (added NO_2 sn.)
7 days later (26 days in all)	,,	no NO_2 reaction	no NO_2 reaction	no NO_2 reaction (asparagin present = ·71 p.c.)

the experiment the original quantity was still present. The same conclusion must be drawn as for the first experiment with ammonia, namely that asparagin inhibits growth but that with a large inoculation of the organism the effect can be largely obviated.

Experiment II. The object was to observe the effect of adding asparagin to solutions in which the oxidation of nitrite had occurred several times. For this purpose two control solutions from Experiment I. in which three successive doses of nitrite had been oxidised, received 2 c.c. doses of a 10 per cent. asparagin solution [giving a concentration of

TABLE VI.

Period of incubation	Control	Asparagin series	
		5 c.c. inoc.	2 c.c. inoc.
6 days	no NO_2 reaction	no NO_2 reaction	no NO_2 reaction (asparagin determined = ·705 p.c.)
2 days later	—	further asparagin added	—
7 days later	no NO_2 reaction	wk. NO_2 reaction	—
3 days later	—	no NO_2 reaction (asparagin determined 1·115 p.c. ·005 p.c. ammonia)	—

0·71 per cent.], together with a fresh dose of nitrite, and were incubated with a control which received no asparagin but fresh nitrite at the same time. The results of this experiment are shown in Table VI. Here there was no slowing of oxidation in the presence of asparagin at the outset, the two cultures containing that substance having oxidised as rapidly as the control, namely in six days. The asparagin was then determined in one of them and found to be identical with the amount originally added. To the other culture a fresh dose of asparagin was added and more nitrite, with the result that oxidation was complete in 10 days as against 7 days in the control. A determination of the asparagin showed 1·115 per cent. and a little ammonia amounting to ·005 per cent. to be present, the latter being due to a slight attack on the asparagin by foreign organisms conveyed in the not perfectly pure inoculation of nitrobacter. One must conclude from this experiment that, when the organism has previously multiplied vigorously in a pure nitrite solution, the subsequent addition of asparagin in amount equal to what had markedly inhibited oxidation in Experiment I., has no effect in checking oxidation.

The same result has therefore been obtained as in Experiment II. of the ammonia series, and the same conclusions may be drawn from the two asparagin experiments as from the Experiments I. and II. of the ammonia series, namely that both substances have an inhibiting influence upon the growth of nitrobacter, but this effect disappears when the growth of nitrobacter is specially vigorous or has become habituated to the inhibiting substance.

The conclusions derived from these various observations on nitrification may be summarised as follows:

1. That carbonates are not the only substances in the soil which serve as bases for nitrification, since a marked nitrification of an ammonium salt can be brought about in the presence of ferric hydrate, either in the freshly precipitated state or as "iron rust." In solutions nitrification is not completed with this substance, probably because the ferric nitrite or nitrate formed dissociates and the solution becomes acid.

2. That neither kaolin nor modelling clay serves as a base for nitrification.

3. That the double ammonium combination formed by the absorption of ammonium salts by modelling clay can most probably be nitrified in the absence of any base, but that the corresponding combination with peat undergoes no nitrification in the absence of a base.

4. That the function of the base in nitrification is to form ammonium carbonate, which is alone directly nitrifiable, and that the facility with which nitrification is set up by different carbonates depends upon the rapidity with which they can react with a neutral ammonium salt to produce ammonium carbonate. This reaction is greater with magnesium carbonate than with calcium carbonate, but is almost absent with copper carbonate, a result which is not due to a poisonous action on the organism.

5. That ammonium salts and asparagin inhibit the oxidation by nitrobacter of nitrites to nitrates, but this action can be largely obviated by

(a) abundant inoculation of the organism;

(b) allowing the organism to multiply before addition of the ammonium salt or asparagin;

(c) inoculating with an organism which has become habituated in previous culture to ammonium salts or asparagin by gradually increasing the concentration of the latter substances.

THE HYBRIDISATION OF CEREALS.

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With Plate I.

THE purpose of the present paper is to give some account of experiments with oats, wheats, and barleys, carried out personally at St Andrews during the past five years.

The Hybridisation of Oats.

The production of new varieties of Oats by crossing is not a very difficult matter. A fair knowledge of floral structure suffices to lead to successful methods of manipulation.

Some experimenters insist that they have succeeded in raising new varieties by simply sowing two distinct varieties together, leaving them to nature, and gathering the grain. If success is ever achieved by this method it can only be as the result of a series of fortunate accidents of very unusual occurrence. The writer's experience leads him to believe that before natural crossing could take place a number of obstacles would have to be overcome. Flowers of the different varieties would have to open simultaneously. The anthers of certain flowers would have to prove abortive, or their pollen infertile. Pollen would require to be carried from the anther of one kind to the stigma of the other. In spite of many obstacles, however, instances of genuine natural crossing of cereals are recorded. Shirreff[1] affirms that a natural cross-bred wheat appeared in his plots. Dr Charles E. Saunders[2], of the Central Experimental Farm, Ottawa, records the finding of a natural cross between Polish wheat and some other variety in a plot of Polish

[1] Shirreff, *Improvement of the Cereals*, p. 46.

[2] C. E. Saunders, *American Breeders' Association*, Vol. I., 1905, p. 137.

wheat. Rimpau[1] in the course of his observations found a number of naturally crossed wheats, barleys, and oats, the wheats being most numerous.

In experimental crossing it is obviously quite essential to remove the anthers at an early stage from the florets to be pollinated, and it is also a wise precaution to protect the prepared florets both before and after the application of the pollen. Small bags are commonly used for the purpose of protection. The writer has found specially constructed glazed boxes convenient. The outer floret of the spikelet was invariably taken for pollination in the present experiments.

The following crosses were successfully carried out in 1901:

Goldfinder with Potato	4 grains secured.
Goldfinder with Waverley	3 ,, ,,
Goldfinder with Black Tartarian ...	2 ,, ,,
Waverley with Black Tartarian ...	1 grain ,,
Black Tartarian with White Canadian	1 ,, ,,
Black Tartarian with Abundance ...	1 ,, ,,

It would serve no good purpose to dwell on details of the failures met with, for it is probable that they were very largely due to misfortune or inexperience. So far as the writer is aware it should be possible to cross any variety of oat with any other if due skill be employed. Nevertheless there are circumstances which tell against success. In 1903 an effort made to cross a considerable number of varieties resulted in complete failure. The non-success was attributed to the effect of over-stimulation by a nitrogenous fertiliser. The season, however, was very unpropitious. It is a well-known circumstance that when plants are in too active a condition of vegetative growth they are unlikely to set fruit well.

The crossed grains, twelve in number, were sown singly in small flower-pots, and when the first green leaf was about an inch above the ground they were planted out. One failed to germinate. The remaining eleven plants grew to unusual size, outstripping the examples of the parent varieties alongside. The latter were grown in a manner quite similar to the hybrids, except that they were sown in the ground direct. The excessive vigour of the hybrids could not be accounted for by any help that transplanting might give; it seemed clearly to be due to inherent qualities, and to afford another instance of the well-known phenomenon of enhancement of vitality consequent on crossing.

[1] Rimpau, *Landw. Jahrb.*, 1891.

Descriptions of Parent Plants and Hybrids.

The hybrids between black and white oats have been found to offer most features of interest. Attention is directed to three of them now, viz. Goldfinder × Black Tartarian, Black Tartarian × White Canadian, and Black Tartarian × Abundance, the remaining one, Waverley × Black Tartarian, being left for discussion later (p. 79).

It will be seen that in the first-named cross the Tartarian is the pollen parent, whereas in the next two the Tartarian is the seed parent. It will be shown in the sequel that so far as the transmission of the colour of the grain goes it is immaterial whether Tartarian occupies the one place or the other. Opportunity was not given of showing whether in the transmission of the form of the ear, etc., a similar state of affairs existed. In the meantime we may pretty safely assume that reciprocal crossing would give identical results. Such has been found to be the case in wheats by other observers. It is by no means the case, however, that all reciprocal crosses are identical, in groups outside of the cereals, at any rate.

Goldfinder.

The parentage of this oat is given by the raisers, the Messrs Garton, as follows: (White Canadian × Yellow Poland) × Winter.

However carefully the new breeds are selected and fixed there must naturally be far greater likelihood of variation in recruits, so to speak, than in veterans which have stood the test of time. The stock of Goldfinder was, it is believed, quite above suspicion of admixture, but the records of the present experiments point to a tendency to variation in the form of the ear and some instability in the varying number of the grains in the spikelet, four being occasionally found. The ears of the plants used as parents were strongly inclined to hang to one side, but could not be classed as other than pyramidal. The grains were golden.

Black Tartarian.

This old standard oat is characterised by two marked features, the one being the unilateral form of the ear, and the other the "black" colour of the "grain," that is, the colour of the pales which enclose the grain proper. The spikelets consist of two florets, a third being occasionally seen. Awns may be numerous, few, or none. The ear is dense, this being due to the upright growth of the branches. When the spikelets interlock, as they usually do, more or less, the ear looks very narrow.

Goldfinder × *Black Tartarian.*

Of this cross one grain was good, the other very poor. The latter was solid enough at its basal (germinal) end, but shrivelled and empty-looking above. Notwithstanding the deficiency in store of starch, the seedling from this poor grain grew fairly well. It bore the appearance of having been checked at the commencement of growth, and it lacked the vigour of its neighbour. Its strongest stem rose to 6 ft. 6 in. There were four other strong stems and 17 weaker ones, besides a crowd of weakling late shoots. The longest ear was 18 inches.

The plant from the good grain bore eight strong stems and nine weaker ones, but no late ones. The tallest attained the height of 6 ft. 6 in., and the longest ear was 18 in. This ear (Fig. 1) was composed of 296 spikelets, and, allowing for grain lost, it was computed to have borne 657 grains. Neglecting the difference in vigour, the two plants were alike in all respects.

The ears showed a blending in form between the two parental types, being somewhat elliptical in outline and considerably flattened one way. The grains also were intermediate, being rich brown in colour. A third grain in the spikelet appeared frequently. Awns were hardly ever developed.

White Canadian.

The grain used under the name of White Canadian was taken from a commercial sample, and its history cannot be traced. There is much reason to believe that this sample contained more than one variety, and therefore considerable uncertainty exists regarding the authenticity of the seed parent. So far as the present enquiry is concerned, the dubiety is not of much consequence. The tallest plant was 5 ft., the ears pyramidal, spreading, the longest one 11 in. Three-grained spikelets occurred, but they were not numerous. Awns were absent.

Black Tartarian × *White Canadian.*

The hybrid grain was small. In spite of this the plant grew well, the tallest stem reaching over 6 ft. Ten strong stems and 12 weaker ones were produced. The longest ear was 18 in. The form of the ear was fairly intermediate, spreading considerably, but decidedly one-sided. A third grain was frequently present in the spikelet. The grain ripened to a rich brown colour. Awns were absent.

Abundance.

The pedigree given by the raisers, the Messrs Garton, is as follows: White August × White Swedish.

The ear of this variety is open pyramidal, and the grain is white. Three-grained spikelets are of common occurrence, and awns are very exceptional. The tallest plant in the plots was 5 ft. 7 in., and the longest ear 11½ in.

Tartarian × Abundance.

The single grain secured of this cross was a good one, and the plant grew well, producing 11 strong stems and 11 weak ones. The tallest reached the height of 6 ft. 4½ in. The longest ear was 15½ in. The ears were somewhat open, but with a marked tendency to the one-sided type. Three-grained spikelets were of fairly common occurrence. The ripe grain was deep brown. No awns were present.

The hybrids were very late in ripening, and when still unripe a gale beat against the frame of the net under which they were placed, bending the majority of the stems and breaking a good many. Immediate steps were taken to straighten and support the bent and half-broken ones, but it was manifest that the crop was lessened very considerably by the accident.

Selection of the Hybrid Seed.

In spite of the damage done by the gale it was found possible to select much fine seed for further cultivation. The system of selection adopted was as follows: The grains were selected in the first place according to their position in the spikelet, whether outer, mid or inner. The grains were taken almost without exception from spikelets composed of three grains. The grains so selected were mixed in their several classes, and sets of hundreds, sometimes fifties, picked out, commencing with the finest samples. Twelve thousand of these grains were sown in dibble holes made six inches apart each way.

The vigour of the hybrids was again in evidence in the fine growth made by the entire crop. Everything went well for a time, but the extremely bad weather of the autumn of 1903 wrought great destruction over the whole plot. The plants were usually over 6 feet high, but they lodged and much grain was thus lost. Harvested in a sodden state, the heads of each plant were tied together, and the product of the rows made into separate bundles and hung up to dry. The ears were

so seriously damaged as to preclude the possibility of making comparative observations on their form. This was a very regrettable loss, because it was obvious that very interesting variation had taken place in respect of this character, by the occurrence in many of the Black Tartarian crosses of examples with black grains and spreading, pyramidal, not one-sided, ears. It was impossible, however, to discriminate between different shades of brown, which in perfect grain might have enabled one to separate such plants as might have taken after the hybrid parent from those which might have resembled the Tartarian grandparent. The so-called "black" of the latter is merely a very deep brown, and well-matured hybrid grain and badly-matured Tartarian grain are of the same tint. It is convenient to designate all that take after the Tartarian grandparent in colour characters as "black," and all that take after the other grandparents—whatever be the shade of gold, straw-colour or whitish—as "white."

Account was kept of the numbers of blacks and whites in the three sets, and the subjoined table drawn up:

Goldfinder × *Black Tartarian.*

(From the two plants of the first generation.)

Grains sown.	Plants harvested.	Black-grained plants.	White-grained plants.	Ratio of black and white.
(1) 1000	567	433	134	3·23 : 1
(2) 900	566	415	151	2·75 : 1
		Black Tartarian × *White Canadian.*		
890	532	379	153	2·48 : 1
		Black Tartarian × *Abundance.*		
600	274	209	65	3·21 : 1.

It is perfectly obvious from the above figures that a fixed numerical relationship exists between the black and white derivatives of hybrid oats in the second generation, in respect of the colour of the grain, the proportion of the former to the latter being very nearly 3 : 1. The totals are sometimes over, sometimes under, the exact terms of this ratio. In all cases the plants saved from the century sets were both black and white. In some cases the black plants from the set were exactly three times as many as the white. In other cases there was a wide departure from this proportion, but when a sufficiently large number of sets were added together the general average approached the simple ratio closely.

In other cereals the characters which have been found by Spillman, Biffen and others to be subject to Mendelian segregation are such as the form of the ear, the colour of the grain, the colour of the chaff, the presence or absence of awns, etc.

If the hybrid oats conformed to Mendel's law it was obvious from the tables given that the blacks should be dominant and the whites recessive. Experiments were proceeded with to prove this.

As already stated, it was impossible to separate the blacks from the browns in the specimens dealt with above. Samples were taken from individual plants (often from single heads). The grains from the blacks were sown in rows of 100 or 50 side by side, and the white derivatives of the same origin were sown in a similar way in the near neighbourhood.

No black oats were to be found among the white ones at harvest, that is to say, the expectation based on Mendel's law was fully realised, and the recessive types demonstrated to be fixed as regards this character.

It was otherwise with the rows of blacks. Here a large number of white-grained plants were found dotted promiscuously in certain of the rows, while other rows were entirely composed of black-grained plants. It was clear that grain from plants repeating the hybrid character had been sown in the former, while in the latter the grain had been from plants which were pure extracted dominants.

Experiments with the Grains of Individual Spikelets.

An effort to test whether the grains of individual spikelets varied among themselves as bearers of hereditary qualities; in other words, whether they would produce either dominant or recessive characters only, or both. This experiment was not tried in 1903 when the bulk of the hybrid grain was sown, but fortunately a few ears—very poor ones—of Goldfinder × Black Tartarian and Black Tartarian × Abundance had been kept. Twelve spikelets of each kind were secured, and these yielded sufficient seed to show at least general results. The spikelets all bore three grains, the innermost (uppermost) one being in most cases very small. The grains were all rich brown.

The following method of sowing was followed:—The grains of each spikelet were sown in series, the largest marked I, the mid one II, and the smallest III. The seed was not only a year old, it was also very late in being sown. The plants made very indifferent growth, and were harvested late in poor condition. It was impossible to say in several

cases whether the grain gathered was black or white, and this doubtful grain could not be counted.

The subjoined table, compiled from the available grain, shows the distribution of colour over the several sets, each plant of course being found to bear either black or white grain:

Goldfinder × Black Tartarian.

Position in spikelet...	I.		II.		III.	
Colour...	Black	White	Black	White	Black	White
Spikelets						
1	×	—	×	—	—	×
2	×	—	×	—	—	×
3	—	×	×	—	—	—
4	×	—	—	×	—	—
5	×	—	—	×	—	×
6	×	—	×	—	×	—
7	×	—	×	—	—	×
8	—	—	×	—	—	×
9	×	—	—	×	×	—
10	×	—	—	×	×	—
11	—	×	—	×	×	—
12	×	—	—	×	—	×
Black Tartarian × Abundance.						
1	×	—	×	—	×	—
2	×	—	—	—	—	—
3	×	—	×	—	—	×
4	—	—	×	—	—	—
5	×	—	—	—	×	—
6	—	—	—	×	—	—
7	×	—	—	×	×	—
8	×	—	—	×	—	×
9	—	—	×	—	×	—
10	×	—	—	—	×	—
11	×	—	×	—	×	—
12	×	—	×	—	×	—

The total number of plants bearing either black or white grains can be tabulated as follows:

Goldfinder × Black Tartarian.

	I.	II.	III.
Black	9	6	4
White	2	6	6

Black Tartarian × Abundance.

Black	9	6	7
White	0	3	2

Poor as the material was it proved to be sufficient to show that in the original hybrid the grains of individual spikelets varied among themselves in respect of the latent characters they were endowed with, and that no account need be taken of the position of the grains in the ear so far as the distribution of hereditary traits is concerned.

The time had now come when it was desirable to grow larger quantities of certain of the varieties. Accordingly selection was made of a varying number of plants that were regarded as alike in the several rows of the third generation, which had been sown from separate hundreds (or fifties) as described. The grain of the plants chosen from a row was mixed together, and a quantity of it sown by hand about 3 inches apart in drills of the usual kind in the field.

The history of a number of the plants of which continuous records had been kept to the fourth generation is summarised in the annexed table:—

	Position of grains in spikelet of 1st generation	No. of 1st generation sown	Segregation of plants of 2nd generation harvested		Single plant chosen for 3rd generation		No. of grains sown	No. of plants harvested	No. of similar plants chosen for 4th generation	No. of sheaves of 4th generation	Character of plants of 4th generation
			Blacks	*Whites*	*Dominant*	*Recessive*					
A	First	100	54	14	—	×	100	78	Many	14	Heavy, drooping, one-sided ears, to be regarded as fixed. Much stronger than Potato, and about a week later.
B	First	100	38	13	—	×	50	43	33	12	One-sided ears; none pyramidal. Held to be fixed. Not early.
C	Second	100	42	17	—	×	50	34	9	5	Fine, tall, one-sided grain. A few pyramidal ears.
D	Third	50	20	5	—	×	50	35	34	25	One-sided, open and close, with a few distinctly pyramidal.
E	Second	100	37	16	×	—	50	24	17	7	Open one-sided, uniform, with an occasional pyramidal.
F	Second	100	42	14	×	—	50	30	25	17	Close or somewhat open one-sided. A few pyramidal. (One white-grained plant.)
G	Second	100	42	14	×	—	100	68	48	17	All pyramidal, spreading or slightly compressed. Held to be fixed.
H	Second	100	54	15	×	—	50	38	29	8	Mixture of one-sided, hybrid and pyramidal.
I	First	100	54	12	×	—	50	29	25	9	Mixture of one-sided, hybrid and pyramidal. Very tall, and extraordinarily late.
J	First	100	43	17	×	—	50	36	24	16	Open one-sided and pyramidal. Not so tall as usual.
K	First	100	53	17	×	—	100	78	60	18	Mixture of close one-sided, hybrid and pyramidal.
L	Second	100	49	19	×	—	100	78	56	8	Open and close one-sided. None quite pyramidal.
M	Third	100	54	12	×	—	100	59	31	12	Mixture of one-sided, hybrid and pyramidal.
	Black Tartarian × White Canadian.										
N	First	100	54	18	×	—	100	75	67	7	The great majority pyramidal. A number of fairly close one-sided ears.
	Black Tartarian × Abundance.										
O	First	100	47	12	—	×	100	51	42	6	Mixture of pyramidal and open and close one-sided. The pyramidal greatly in the majority.
P	Second	100	18	7	×	—	100	70	64	6	All pyramidal and quite uniform.

The above table is interesting in showing clearly the difficulty in selecting when one has not been fortunate enough to get a full knowledge of the Mendelian characters of the plants worked with. So far as colour of the grain is concerned, the only point to notice is that one white-grained plant appeared in F, the bulk of which amounted to seventeen sheaves. It is in the highest degree probable that a single white seed had been sown amongst the black by accident.

In A and B the results are white forms with a one-sided ear, now fixed. In C and D, also white forms, there seems to be still a tendency to vary, a few pyramidal ears appearing amongst the one-sided forms. G and P are examples of black varieties with pyramidal ears, now fixed. In the other varieties there is ample room for further selection.

In I a feature of unusual interest was noted. All the plants, regardless of the form of their ears, were absolutely uniform in one particular, viz. distinct lateness. The row had to be left to ripen when all the others had been harvested.

In one of the experiments with Tartarian × Abundance, the line of descent of the plants was different from that followed by those in the above table. To begin with, four rows of grain from the original hybrid yielded the following:—

	Blacks	Whites
A	18	7
B	49	9
C	33	17
D	47	12
	147	45

Grain from a single plant of each of the "blacks" of A, B, C, D was chosen and sown, and the following plants harvested:

	Blacks	Whites
A′	63	20
B′	30	11
C′	27	10
D′	22	8
	142	49

The totals in both tables show a close approximation to the Mendelian ratio. The second table points unmistakably to the fact established by Mendel that certain of the progeny of the third generation repeat the dual character of the second, and do not breed true. The grains of A′, B′, C′, D′ were rich brown, and that feature in itself betokened their hybrid character.

The whites from A′, B′, C′, D′ were mixed and sown in the field so as to yield eight sheaves. It was found that about a dozen plants with black grains and pyramidal ears appeared in the lines. Their presence could only be accounted for by accidental introduction of black seed. Although great care had been exercised to prevent mixture, it was not impossible that a bleached grain or two might have escaped notice in the course of selection. There is no reason at all to suppose that the route taken in descent had anything to do with the appearance of the blacks amongst the white recessives.

Waverley × Black Tartarian.

As already stated, this hybrid has not conformed to rule. The hybrid could not be distinguished from Waverley unless by its greater height and general vigour. The open pyramidal form of the ear was the same. The best ear was 13 in. long, and the height of the plant 6 ft. 5¼ in. The grain of this plant was sown along with that of the hybrids above described. The result was remarkable. The plants saved from the wreckage of 1903 showed that a trace of the blood of the Black Tartarian was in their constitution, for a distinct black form appeared amongst the normal Waverley-like plants. No other types appeared except these two. The black one had a spreading pyramidal ear. It was represented by remarkably few plants. Many examples of both types were noted to be over 6 ft., the tallest in the normal form being 6 ft. 3½ in., and in the black 6 ft. 5½ in.

The number of Waverley-like plants lifted was not recorded, but all the black ones were carefully selected out and counted. Judging from the average number of plants of the other crosses saved and counted, it may be safely conjectured that the number of both lifted would not be less than one-half of that sown. On this basis the subjoined table has been framed:—

Waverley × Black Tartarian.

Proportion of Black-grained to White-grained Plants.

Position of grains in spikelet	No. of grains sown	Minimum no. of plants saved (conjectural)	Actual no. of blacks present
Outer	700	350	10
Mid	300	150	5
Inner	200	100	3
Totals	1200	600	18

Keeping in mind that both whites and blacks suffered diminution in the same degree, it is obvious from the above table that the number of black derivatives does not approximate at all to the Mendelian ratio. It may be described as a case in which the pollen parent has been for some unexplainable reason almost but not quite impotent.

The production of black-grained plants from all three grains of the spikelet is a point of interest in connexion with what has already been noted regarding this matter.

Next year 350 white grains were sown in the plots. At harvesting there were probably about 250 lifted, and all were of the original Waverley-like type except *two*, which were of the pyramidal black type.

In the following year (1905) such a number of white grains was sown as to yield eight or nine sheaves at harvest. *No example* of the black form was found in the lot.

Grains of the black oats were sown in the plots. Of the 53 plants harvested 34 were found to be of the black type, and the remaining 19 the white type. The white plants were conspicuously the stronger. No one-sided examples of either type were found. The number grown was scarcely large enough to ensure statistical certainty, but at present one may be justified in assuming that the black forms derived from the white ones in very small numbers, themselves give off white forms in relatively large numbers. The suggestion of the possibility of the black-grained plants being in this case of the nature of recessives is thus done away with.

Referring in conversation to the peculiarities of the above hybrid, Mr John Garton informed me that he had met with anomalous cases in some measure corresponding to the above. All who have had long experience in crossing must have seen instances which are exceptions to the rule. It is well to remember that the plants we work with are not built up after all with mathematical regularity, but that they are the outcome of developmental processes acting in inconceivably diverse ways and through long ages of time, and that therefore there need be no surprise if they do not always fall in with the rigid and narrow formulae of the statistician. The practical lesson to be learned from this case is that it is possible that good things may be lost by too close adherence to arbitrary rules of selection.

Experiments in Crossing Wheats.

Several crosses were carried out with wheats, viz., Red King × Red American, Red King × Scotch Bethlehem, and Red King × Rood

Koren. The most interesting of these is the last mentioned and it alone will be described.

Red King.

The raisers of this wheat, the Messrs Garton, give the following as its pedigree:—(Lincoln Red × Michigan Bronze) × Waterloo. The straw of this variety is of medium length and particularly strong. The ears are long and compact (Fig. 2, *a*).

Rood Koren.

This bearded wheat (Fig. 2, *c*) was received from a friend who stated that it had come from the Orange Free State. Request for information being made regarding it to Mr William Macdonald, M.S., Agr., Editor of the *Transvaal Agricultural Journal*, the following particulars have been kindly given by him:—

"The Rood Koren was imported eight or ten years ago from Canada by Mr A. C. Macdonald, Assistant Director of Agriculture, and is probably a strain of Red Egyptian. It is a very hardy variety, and is widely used for milling purposes. Besides being considered one of our very best flour wheats, it is more or less rust-resistant."

Mr J. Burtt-Davy, Agrostologist and Botanist to the Transvaal Department of Agriculture, obligingly adds the following note:—"I may point out that of some 200 varieties of wheat tested by us last season, only one proved rust-resistant. This was the 'Red Egyptian,' obtained from Vilmorin-Andrieux et Cie, Paris."

The Rood Koren grew from 2 to 3 feet high in the plots. Some examples in special ground were nearly 4 feet. The length of the ears is $2\frac{1}{4}$ to $2\frac{3}{4}$ inches, or including awns about 5 inches. The awns are from $2\frac{1}{2}$ to $3\frac{1}{2}$ inches. The average number of grains in the ear is 40. The greatest number of ears produced by one plant was 28.

Rood Koren has been singularly free from rust in the plots, and its progeny have been far less subject to the disease than those of the other crosses named above.

Red King × *Rood Koren.*

Four grains were secured of this cross in 1902. All four grains were from one ear of Red King. They were sown in the ground in November of the same year. One, a poor grain, failed to germinate, the other three grew apace. On July 2nd, 1903, it was noticed that

while two plants of the pollen parent (Rood Koren) were in flower, and all the plants of the seed parent had their ears still hidden within the sheaths, all the ears of the three hybrid plants were clear of the sheaths. The hybrids thus showed an intermediate condition in respect of the period of shooting into ear.

The hybrids were harvested about Oct. 6th. All three were identical in general characters. The tallest of each were as follows: A, 4 ft. 4 in.; B, 4 ft.; and C, 4 ft. 4½ in. The number of ears respectively were 19, 21, and 30. The longest ears in all were 3⅝ in., a common length being 3 in., and the longest awns (projecting from the apex of the ear) 1 in. The ears were square and compact (Fig. 2, *b*), and the straw much more slender than in Red King.

The average numbers of grains for the ten best ears of each were as follows: 54·9, 58·6, 62·3, giving a common average of 52·6.

Many grains of each were sown on 11th Dec., 1903. During the following summer it was again noticed that the hybrids showed an intermediate condition in the time of flowering, in many cases at all events.

The plants of the second generation showed the usual breaking up into many forms. Four distinct types could be easily recognised, viz. short-eared, long-eared, awnless, and awned (Fig. 3). When harvested the progeny of each original plant, A, B, C, was looked over and the awnless separated from the awned; then the same plants were looked over again, and, regardless of the awned or awnless condition, the short-eared separated from the long-eared. The subjoined table shows the proportions of the respective stocks, and the totals for all three:—

Red King × *Rood Koren.*

	Awnless	Awned	Short-eared	Long-eared
A.	164	58	165	57
B.	255	86	240	101
C.	245	63	238	70
Totals	664	207	643	228

The totals point unmistakably to Mendel's ratio of 3 : 1, the awnless being almost three times as many as the awned, and the short-eared occupying a similar relationship with the long-eared. These results are corroborative of previous ones got by other observers. The awned and the long-eared being recessive characters, one expects varieties possessing them to breed true to these characters.

It was noticed that there was very little variation indeed in the form of the ear of the long-eared types, both awned and awnless falling into one class. It was otherwise with the short-eared types. These could with considerable accuracy be classed into two sub-groups—longer and shorter. The following table, framed from the material available, shows the proportion of these types, their awned or awnless condition being taken into account:

Short-eared Types.

	With awns		Without awns	
	Longer	Shorter	Longer	Shorter
A.	30	13	78	44
B.	40	10	62	20
C.	34	19	188	67
	104	42	328	131

The totals here seem to point to the existence of Mendelian proportions in an inner circle. It would be unsafe, however, to press the matter further, the distinction to be drawn between the two sections—longer and shorter—not being very clearly defined.

A few plants of the short-eared type bore remarkably dense ears.

The grain from the three sets, A, B, C, was separated with reference to the length of ear and the presence or absence of awns, and the parcels under the respective heads mixed together for sowing. The examination of the crop showed that the Mendelian anticipations were fully realised. The plot containing the lot which embodied the two recessive characters, long ears with awns, presented the most uniform appearance, the ears being almost entirely long and awned. In the plot sown with grain from long but awnless ears, the ears were almost all long, and the majority were awnless. The plot sown from short-eared awned specimens was composed virtually of entirely awned plants, but the form of the ears was exceedingly variable, few, however, being long. The plants derived from the short awnless ears were of all types, awned and awnless, long and short, the long-eared and the awned being greatly in the minority.

Grain from a single short awnless ear was sown in a row by itself and the crop was found to be composed of every type, thus giving a complete repetition of the forms which occurred in the second generation. This result clearly emphasised the fact that the short awnless plants retain the hybrid characters and do not breed true.

The Hybridisation of Barleys.

The experiments on the crossing of barleys were commenced in 1903. Standwell was chosen as seed parent and crossed with other varieties of barley, viz. (*a*) a six-rowed variety which appeared amongst the Rood Koren wheat in the plots, and for convenience now designated Stranger, (*b*) Zero, (*c*) Egyptian, and (*d*) Bere.

Standwell Barley.

This variety (Fig. 4 *et seq.*, *a*), as stated by the raisers, the Messrs Garton, is a cross between Fan and Golden Melon. It is two-rowed, strong-growing, and early.

Zero Barley.

This interesting hybrid, also sent out by the Messrs Garton, has the following pedigree: (Winter × Fan) × Swedish. It is six-rowed and a distinct variety in every way. At an early stage of growth the foliage is distinctly dwarf, compared with either Standwell or Stranger, and it is the latest of all the varieties at present in question in showing ear.

Six-rowed Barley (Stranger).

This variety (Fig. 4, *c*) came up in the experimental plots, and was possibly brought from the Transvaal with the Rood Koren wheat. It bears much resemblance to Zero, but its fresh foliage, while of the same shade of light green, is much stronger and taller, and it is somewhat earlier in showing ear.

Egyptian Barley.

This old variety is a two-rowed form, and in that respect is to be classed with Standwell, but it has a well-known peculiarity in that many of the plants bear ears which are more or less branched (Fig. 6, *c*). In extreme cases the branching may give rise to tufted ears. The specimen used as pollen parent was only slightly branched. The foliage of Egyptian and Standwell is of the same dark green, that of the former being slightly but appreciably less strong than that of the latter. Standwell is slightly earlier in showing ear.

Bere.

The ear (Fig. 5, *c*), actually six-rowed, has the appearance of being four-rowed. It is large, drooping, and extremely strongly awned. Clear

rose-purple lines beautify the exterior of the outer pales (flowering-glumes). Bere shoots into ear, flowers, and ripens considerably earlier than any of the barleys above described.

Only a few crosses were attempted, and the successful ones yielded the following grains: 4 as the result of the cross with Stranger, 3 with Zero, 1 with Egyptian, and 1 with Bere.

The grains were sown in the open. Two of the Stranger cross and two of the Zero cross died off, and the Bere cross was attacked at the root by some pest and was seriously checked. The remaining plants having plenty of room, the tillering in most cases was remarkably free. The hybrid nature of the plants was unmistakable in all cases.

Opportunity was missed of noticing the relative earliness of flowering of the hybrids. Their ears were more or less intermediate in character between the parental forms. The ears of the cross with Zero bore very little resemblance to those of the cross with Stranger (Fig. 4, *b*), the former having a marked leaning to the six-rowed type, while the latter had an equally marked leaning to the two-rowed type.

The number of the grains in individual ears have not been noted, but the following enumeration of grains taken from the single hybrid plants will serve to show their productiveness.

	Stems	Good ears	Tallest	Grains in ears	Average in each ear
Standwell × Stranger ...	54	39	4 ft.	673 in 30	22·4
,, ,, ...	33	14	4 ft.	287 ,, 12	23·9
,, Zero	32	21	3 ft. 9 in.	451 ,, 18	25
,, Egyptian	36	36	3 ft. 8 in.	820 ,, 29	28·2

The figures for the cross with Bere have not been put down, normal development having been checked. Four or five good ears of it were saved, and 82 grains secured for sowing.

The seed of all four hybrids was sown in lines in 1905. The first feature of interest noted was the evidence of the impression of the early character of Bere on its hybrid progeny. When the ears of Bere were almost clear of the sheaths, and those of Standwell were still quite enclosed, several of the hybrids showed ears projecting considerably from the sheaths. It soon became obvious that there were three marked types in the lines, those resembling either Bere or Standwell, and the hybrid forms. The early character of Bere was seen to be impressed

on a certain number of all three types, and it was interesting to see the plots at the stage when the Bere and its early hybrid progeny were in the ascendant, their ears alone being visible and projecting a foot or more above all the other barleys, both hybrid and parent, in the plots.

Bere was the first of the series to flower. It was followed next day by one of the progeny having marked hybrid characters. These were quickly followed by others which showed a strong leaning to either one or the other grandparent.

At about the time this stage was reached it was found that when 15 of the plants had their ears clear of the sheaths, 51 of them were showing various degrees of retardation, from the latest with the tops of the awns projecting only half an inch beyond the sheaths, to conditions approaching release of the ears. Although the line of demarcation between earlies and lates was not very strong, it was impossible to help thinking that the early forms might prove to be recessive and so be readily fixed.

It may be mentioned that the plants of the Zero cross were somewhat later than those either of the Stranger or the Egyptian cross. Earlier and later forms appeared in both the Zero and the Stranger crosses, whereas the Egyptian cross was very uniform in respect of its flowering period.

Standwell × Bere.

At harvest it was found that the plants of this cross could be divided into two series, two-rowed and six-rowed. The aggregate of the six-rowed, including the hybrid forms, was 55, and of the two-rowed 14, an approximation to the Mendelian ratio which seemed to warrant the assumption that the two-rowed character in this cross is recessive.

Two or three plants were found to be indistinguishable from Bere, even to the presence of the rose-purple lines on the pales. On the other hand, several bore a very great resemblance to Standwell, the only difference being that the grain of the former was not so plump as that of the latter. A number of the hybrid forms could perhaps be classed as identical with the hybrid parent (Fig. 5, *b*), a greater or less number of the usually sterile florets being fertile, but with small grains; and these forms were connected with the others closely resembling Bere or Standwell by many stages of variation. For instance, all stages seemed to be represented between the extremely strongly-awned pales and fully-developed grains corresponding to the extra (lateral) ones in Bere, through samples with weaker-awned pales and smaller grains in that

position, to types like Standwell in which both grains and awned pales were absent there.

Examples occurred in which the grains of all the six rows were fully developed, but the lateral ones were without awns. The awns present in the central grains in such cases varied in strength, being sometimes as strong as those of Bere.

Plants with ears of a remarkably long, open or lax character occurred in both two-rowed and six-rowed types.

Standwell × Stranger.

The plants of this cross were easily separated into two series, two-rowed and six-rowed. Of the former there were 165 plants, and of the latter 52. It seems quite certain that these figures satisfy Mendelian expectations, and that the six-rowed character, as has already been shown by other observers[1], is here the recessive one.

A very remarkable similarity of form existed amongst the six-rowed plants, and they differed from Stranger in being somewhat shorter in the ear. A few plants, however, had ears of the same size. It is possible that exact repeats of Standwell may have occurred, but such were not noticed.

The two-rowed plants were also much alike among themselves, and they differed from Standwell in the ear being somewhat longer and narrower. A further difference lay in their possessing or retaining very short awns on the pales of the lateral florets, whereas in Standwell these have disappeared and left the pales truncate at the apex.

One or two plants showed the hybrid characters more strongly in possessing lateral grains with awned pales, but both more or less poorly developed. On the other hand, examples with truncate pales occurred in a few of the hybrid forms. Nine or ten plants were found with particularly lax ears.

Standwell × Egyptian.

Time did not permit of a full and careful inspection of the plants of this cross. Less than half of the material was looked over. In the 251 plants examined, 208 were set down as normally two-rowed, and 43 as more or less of the Egyptian type.

It is highly probable that many having the Egyptian character

[1] See Biffen, "The Inheritance of Sterility in the Barleys," *Journ. of Agric. Science*, Vol. I. Part II.

inconspicuously developed would be placed in the larger bundle. In this connexion it is to be remembered that unbranched plants occur in plenty in fields of genuine Egyptian. Further, it is to be noted that, in selecting, both branched and unbranched ears were found on one and the same plant.

The original plant was, as is already mentioned, only slightly branched. Many of the present lot were more branched than it was, but comparatively few were very much branched.

The plants of the normal two-rowed lot were like Standwell, but there was some variation in the form of the ear, some of the ears being much longer and opener than those of Standwell.

Standwell × *Zero.*

Opportunity was missed of examining the plants of this cross after harvest. This is to be regretted, because it would have been interesting to compare them with the plants of the Stranger cross.

The writer has to acknowledge assistance from the Carnegie Trust for the Universities of Scotland, during the last two years of his experimental work.

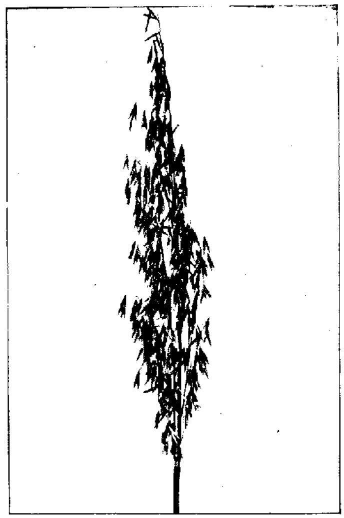
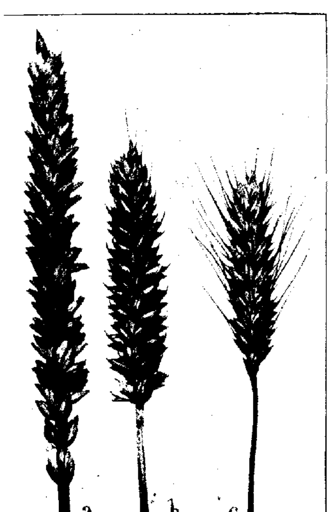


Fig 1.—Ear of Goldfinder × Black Tartarian Oat.



2.—Ears of *a*, Red King; *b*, Hybrid; , Rood Koren Wheat. Reduced.

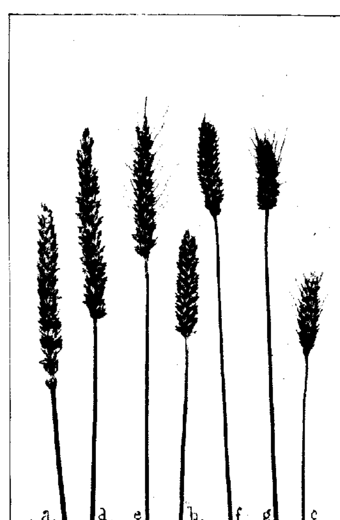


Fig. 3.—Ears of *a*, Red King; *b*, Hybrid; *c*, Rood Koren; *d*, *e*, *f*, *g*, four types derived from the hybrid.

Fig. 4.—*a*, Standwell; *b*, Hybrid; *c*, Stranger Barley. Natural Size.

Fig. 5.—*a*, Standwell; *b*, Hybrid; *c*, Bere. Natural Size.

Fig. 6.—*a*, Standwell; *b*, Hybrid; *c*, Egyptian. Natural Size.

THE HEAT VALUE OF MILK AS A TEST OF ITS QUALITY[1].

By JOHN MALCOLM, M.D., AND A. A. HALL, M.A., M.D.

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An indirect method of estimating the heat value of food stuffs, such as milk, is to determine the amounts of fat, sugar, and proteid chemically, and to multiply by the average caloric value of each; but since the amounts of these substances themselves indicate the quality of the milk their calculated caloric value need not be employed as a test of its quality.

On the other hand, a direct estimation of the caloric value by combustion in an accurate calorimeter might be expected to give an exact gauge of the quality of the milk, and this at one operation occupying a comparatively short time.

The following experiments were undertaken in order to determine how far this direct calorimetry might be depended upon as a test of the adulteration of a sample of milk, when used alone or in conjunction with other data.

Methods. The instrument used in our estimations was a bomb calorimeter (Berthelot-Mahler). The interior is enamelled, except the inside of the lid, which is lined with platinum; the inlet tube for the oxygen acts also as the conductor for the electric current which ignites the substance.

The combustions were performed in oxygen at 23 atmospheres pressure. The "water equivalent" of the bomb, mixer, calorimeter vessel and thermometer (= 323 c.c.) was estimated by the method advised by Stohmann[2], and each 1° C. rise of temperature of the water

[1] The greater part of the work summarised in this paper was done by Dr Hall and presented by him for the M.D. degree as a thesis, for which he was awarded a gold medal.

[2] *Journ. für prakt. Chemie*, 39, S. 524, where much valuable information regarding the bomb calorimeter may be obtained.

placed in the calorimeter represented 2500 calories. The thermometer was graduated in hundredths of a degree C. and with a magnifying-glass could be read to one five-hundredth of a degree.

The correction for radiation (+) was calculated from the observed rate of rise and subsequent fall of temperature; owing to the rapidity of the combustion it seldom rose over ·02°.

A further (–) correction was made for the iron wire oxidized in the ignition and for the union of water with the acids formed by oxidation of the sulphur, nitrogen, phosphorus, etc. At first Kellner's method was followed in preparing the milk for combustion, *i.e.* the milk was dropped on to a known weight of cellulose, and dried. The following is an example of the results obtained:

Cellulose block (0·7286 grm.) saturated with 2·5 c.c. milk, dried at 50° C.

Water 2500 c.c.: rise of temperature 1·743° plus correction for radiation (0·016° C.) = 1·759°; giving 1·759 × 2500 = 4397 calories: minus correction for iron wire used for ignition, and acids formed (22 calories) = 4375.

Average caloric value of cellulose used = 4182; ∴ deduct for cellulose (0·7286 × 4182) = 3047.

2·5 c.c. milk = 1328 calories = 531 calories per c.c.

The disadvantages of this method are very apparent. Only a small amount of milk can be experimented upon, for if the block be once saturated with milk and dried, it does not readily take up a second quantity owing to the dried fat forming a layer on the surface, and the correction for the cellulose is larger than the figure obtained for the milk itself.

After trying various other methods on Kellner's principle we fell back on simply drying the milk at 50° C.[1] in a shallow platinum vessel of about 12 c.c. capacity, which could be placed in the position in the bomb usually occupied by the carrier for the substance to be incinerated. The milk generally dried to a thin superficial scale with a deeper layer on the bottom of the dish, and the ignition wire (iron) was either placed so as to touch this, or a cotton thread, placed partly in the milk previous to drying, was made to hang over the wire and to act as the carrier of the igniting spark.

10 c.c. milk was taken each time, but the results are given for convenience as so much per c.c.

[1] The drying was not complete; we generally aimed at having 5 to 7 °/₀ moisture in the dried residue, as that amount is usual in estimations of the heat value of food stuffs.

As shown in the control experiments (Table I), and in the comparison of direct and indirect methods of estimating the caloric values (Table IV), the method gave accurate results.

I. *Comparison of Heat Values of the same and of different Samples of Milk.* (Table I.)

In this, as in all the other cases, samples of the milk were obtained from various dairies in Edinburgh, and "sweet milk" was asked for on each occasion. In doing controls of any one sample of milk dilutions with water were employed so as to test the accuracy of the method with development of different amounts of heat. (The form of bomb calorimeter used is said to be most accurate when the amount of heat involved is about 7000 cal.)

TABLE I.

	Calories found per c.c.	Calories expected per c.c.	Differences in calories per c.c.
Milk No. I.	676	—	
Same diluted with an equal amount of water	337	338	1
Milk No. II.	753·5	—	—
Same diluted with an equal amount of water	379·4	376·7	2·7
Milk No. III.	652·8	—	—
Same diluted with 10 °/₀ water	581·7	586·8	5·1
Milk No. IV.	877·4	—	—
Same diluted with 7½ °/₀ water	813·0	811·5	1·5
Milk No. V.	776·0	—	—
Same diluted with 8 °/₀ water	726·0	717·6	8·4
Milk No. VI.	650·0	—	—
Same diluted with 4 °/₀ water	623·6	624	0·4
Same diluted with 8 °/₀ water	585·4	598	12·6
Milk No. VII.	868	—	—
Same diluted with 5 °/₀ water	829·4	824·6	4·8
Same diluted with 10 °/₀ water	784·7	796·2	6·5

These results are given in the order in which the specimens of milk were examined and include all that were done at that time (with two exceptions in which some unconsumed carbon was found, in which, therefore, the combustion had been incomplete).

The estimations were done by one of us (A. A. H.) who had very little previous experience of the calorimeter; they show, therefore, that accuracy in this kind of work can be readily acquired.

The table also exhibits the efficiency of the calorimeter over a wide range of heat developments (from 3370 cal. in Milk I (diluted) to 8246 cal. in Milk VII). The highest difference between observed and expected calories was 12 cal. per c.c. in VI (diluted with 8 °/$_0$ water). Taking one grm. of fat as having a caloric value of 9318 cal. (Rubner), this number of calories represents ·0014 grm. fat, so that the maximum error in these estimations is a small one, compared with the error in fat estimation by weighing.

II. *Relationship of Fat percentage to Caloric Value.* (Table II.)

Here the caloric value was determined as before, single estimations only being done. The fat was estimated by Adam's method (5 c.c. milk dropped on to fat-free paper, extracted with ether in a Soxhlet apparatus, and the extract dried and weighed); controls were done in each case.

The following results were obtained in milks from various dairies: they are arranged in order of fat percentage.

TABLE II.

No. of Milk	Percentage of fat	Total calories per c.c. by calorimeter	Calories per c.c. due to non-fatty substances
VIII	5·4	888	383
IX	5·4	868	363
X	5·3	829	336
XI	5·1	848	373
XII	4·9	784	328
XIII	4·7	776	338
XIV	4·3	726	326
XV	4·0	690	318
XVI	3·9	663	300
XVII	3·8	682	328
XVIII	3·3	683	376
XIX	3·1	675	386
XX	3·0	628	349
XXI	2·9	601	331
XXII	2·8	607	346
XXIII	2·7	652	401
XXIV	2·6	566	324
XXV	2·5	570	338
XXVI	2·1	540	345

These figures show that there is, as one would expect, a general relationship, but no constant ratio. This results from the fact already observed by Vieth and others that milks of high fat percentage do not have a correspondingly high sugar and proteid percentage. In other words, the "solids-not-fat" is a fairly constant figure in milks, and this is also brought out in Table II, last column. We have here deducted the calories due to fat from the total calories (assuming 1 grm. fat = 9318 cal.). The figures are fairly even throughout the series, showing that the lower fat percentages were probably skimmed or separated milks, while in the milks with high fat percentages the increase in caloric value was mostly due to the increased fat. (In Milk XXIII there has probably been some mistake, either in fat estimation or in calorimetry.)

The heat value which corresponds to a milk containing 3 per cent. of fat is over 600 calories, and probably 650 would make a fair figure for a legal standard.

If that were the standard, a dishonest milk-seller would find it difficult to dilute a rich milk and yet escape detection: suppose he diluted Milk VIII so as to reduce the fat percentage from 5·4 °/₀ to 3 °/₀ he might still escape under the present legal standard, but the caloric value would be

$$\left(\frac{3}{5{\cdot}4} \times 888\right) = 493 \text{ cal.},$$

sufficiently below the standard caloric value for a clear verdict.

III. *Relationship of Total Solids to Caloric Value.* (Table III.)

This was next investigated on similar lines.

The solids of 10 c.c. milk were weighed after drying to constant weight.

The average given by these figures, 12·4 °/₀ solids, equivalent to 720 cal. per c.c. or 5800 cal. per gramme, is higher than would be given by a milk which just satisfies the present legal standard. That standard requires 3 °/₀ fat, and 8·5 °/₀ solids not fat: by deducting 0·7 for ash and dividing the remainder in the proportion given by Vieth for proteid and sugar in cow's milk, we obtain—sugar 4·6, proteid 3·2, and this gives a theoretical value for the caloric value of total solids of about 5650 per gramme. The last column gives the result of applying this to the figures obtained for total solids, and it will be noticed at once that while the rich milks give more calories than one would

TABLE III.

No. of Milk	Solids %	Calories per c.c.	Calories expected if 1 grm. solids = 5650 cal.
XXVII	14·4	868	813
XXVIII	14·2	896	802
XXIX	13·0	812	734
XXX	12·0	682	678
XXXI	12·0	650	678
XXXII	11·8	675	666
XXXIII	11·2	607	632
XXXIV	10·9	572	615
Average	12·4	720	

expect, owing to the increased solids being due to fat, the poor milks show a much lower heat value than expected from the total solids, *e.g.* the solids in XXXIII and XXXIV must have been very poor in fat.

We consider this an important means of detecting removal of fat from milk, and the estimation of the total solids can be readily carried out along with the estimation of heat value.

IV. *Complete Analysis compared with Caloric Value.* (Table IV.)

The methods employed here were the usual ones. *Proteid* was estimated by determining the amount of nitrogen in the precipitate obtained with Almén's solution, and multiplying the result by 6·37; *lactose*, by titration with Fehling's solution after removal of the proteid by acetic acid and subsequent boiling; *fat and caloric value* as before; the *ash* by slow incineration of the total solids.

None of these figures have been brought into the other tables, but a consideration of them confirms the previous work. Thus the accuracy of the calorimeter is shown by the correspondence between the last two columns where the caloric value is given by the calorimeter and also by calculation, using the factors 9318 for fat (Rubner), 5860 for milk proteids [average of 5855 (Danilewsky), and 5867 (Stohmann)], and 3950 for lactose (Rubner). The absence of a constant ratio between fat percentage and caloric value is evident. The estimation of the calories expected from the total solids again shows that the milks with

TABLE IV.

No. of Milk	°/₀ Total solids	°/₀ Lactose	°/₀ Proteid	°/₀ Fat	°/₀ Ash	Calories estimated (per c.c.)	Calories calculated (per c.c.)
XXXV	10·83	4·25	3·08	2·66	·73	572·0	590
XXXVI	14·40	4·82	3·40	5·40	·74	888·5	892
XXXVII	10·82	4·18	3·26	2·50	·76	570·8	589
XXXVIII	11·76	4·48	3·34	3·10	·77	669·4	661
XXXIX	11·08	4·23	3·08	2·88	·76	607·7	614
XL	11·96	4·72	3·36	3·01	·73	656·7	660
XLI	9·79	3·75	3·13	2·11	·72	531·5	528
XLII	12·01	4·21	3·06	3·82	·74	682·7	701
XLIII	13·10	3·80	3·49	5·10	·75	812·6	830

low solids were poor in fat. For example, XXXVII with 10·82 total solids ought to have yielded over 600 calories, whereas only 570 were obtained, and analysis also bears this out (2·5 °/₀ fat).

Summary. The suggestion is made that the direct determination of the caloric value of milk would be an important aid to analysis in the detection of removal of fat from milk or the dilution of milk with water. The operation requires little chemical knowledge, is easily learned, is very accurate and occupies a relatively short time (forty minutes or less after the milk has been dried). After the initial expense of the calorimeter (about £35) the running expenses are light (about sixpence per combustion).

The legal definition of milk might be improved by the adoption of a minimal caloric value per c.c. or per gramme of total solids.

We consider our estimations too few for determining what that standard should be, but in order to correspond with the present legal standard it should not be below 650 cal. per c.c. or 5650 cal. per gramme solids.

The expenses of this research were borne by the Moray Fund for the Endowment of Research in Edinburgh University.

ON THE EVOLUTION OF GAS DURING CHURNING.

By R. D. Watt, M.A., B.Sc., *Carnegie Research Scholar, Rothamsted Experiment Station.*

It is well known that at the beginning of the churning process a considerable volume of gas is evolved, necessitating some method of ventilation of the churn. In old-fashioned churns the gas was allowed to escape by simply withdrawing the cork or plug, while all modern churns have a special arrangement for the purpose. The gas set free has been stated to be carbon-dioxide, but hitherto no definite proof of its source or estimation of its amount has been recorded. It cannot very well be that the gas is formed by any chemical reaction at the time the churning process is going on. It seems more probable that it is produced by bacterial action during the ripening of the cream, held in a state of super-saturation by the liquid and released by the shaking in the churn in the presence of air. This view would account for the fact that it is only during the first hundred or so revolutions of the churn that any quantity of gas is evolved. It was with the object of demonstrating the correctness or otherwise of this latter theory that the following experiments were carried out.

The apparatus used for determining the amount of carbon-dioxide in each case was a simple modification of that for determining small quantities of carbonates in soils described by Mr Arthur Amos, B.A., in the first volume[1] of this journal. The condenser was found unnecessary, and, as no acid has to be added, the thistle-funnel was also dispensed with. The glass tube leading from the first Reiset apparatus (A) was made to extend almost to the bottom of the Jena flask (B), so that air free from carbon-dioxide could be bubbled through the cream, thus assisting in the expulsion of the gas.

As a preliminary to the determinations with cream the amount of

[1] Vol. I. Part 3, p. 322.

CO_2 present in three samples of milk freshly drawn from the same cow on three successive mornings was ascertained. To ensure accuracy the Jena flask and the rubber tubing in connexion with it were detached, the latter being secured by clips. This piece of apparatus was weighed and a quantity of milk (about 300 c.c.) was milked directly into the flask, which was immediately corked up. The whole was then weighed again, the difference giving the weight of the milk. The connexions were made with the two Reiset towers and the pump started, the clip nearest the pump being removed first, so that the carbon-dioxide liberated from the milk during transit was all absorbed by the sodium hydrate in the second Reiset (C). The milk was then gradually heated to the boiling-point and the titration carried out in the usual way.

The amount of carbon-dioxide found was as follows:

1st morning	... ...	78·18 c.c. per litre
2nd ,,	... ...	79·22 ,, ,,
3rd ,,	... ...	77·00 ,, ,,

One quart of cream extracted by the separator from the milk of a herd of Jersey cows was obtained on four separate occasions.

(1) 200 c.c. of this were tested for CO_2 by the above process soon after separation.

The remainder was allowed to stand for 3 to 3½ days at a temperature of from 15° to 17° C., at the end of which time it was in the usual condition for churning.

(2) 200 c.c. of this were taken and the amount of carbon-dioxide present ascertained.

Other 200 c.c. were placed in a large bottle with about 100 c.c. of water, the resulting temperature being about 17° C. (62° F.). The bottle was shaken vigorously in imitation of churning, the evolved gas being allowed to escape by withdrawing the cork a few times.

(3) The amount of CO_2 remaining in this partially churned cream was also determined, the results being shown in the following table:

No. of determination	No. of c.c. carbon-dioxide found per litre of cream		
	(1) after separation	(2) after ripening	(3) after partial churning
1	51·35	233·25	80·35
2	37·95	283·50	51·35
3	39·05	246·50	54·70
4	27·90	142·85	45·75

At ordinary temperatures pure water, or such a dilute solution as forms the serum of cream, dissolves about an equal volume of CO_2 when saturated in contact with pure gas. On exposure to air, however, almost the whole of this gas will come out of solution in accordance with Dalton's law of partial pressures, the amount remaining dissolved being proportional to the percentage of carbon-dioxide in the gaseous mixture now in contact with the liquid. From such a saturated solution, however, the CO_2 only escapes very slowly unless the liquid be agitated; brisk shaking will cause it almost immediately to assume its new condition of equilibrium with the air. In the case under investigation we see that the cream after ripening is considerably supersaturated with carbon-dioxide, so that about 200 c.c. per litre of cream are instantaneously liberated by shaking, before the solution adjusts itself to the mixture of air and carbon-dioxide that is formed within the churn. In an ordinary small churn for churning about four gallons of cream the volume of carbon-dioxide set free would be about 3½ litres, and this, together with the slight rise of temperature, seems quite sufficient to account for the need for ventilation.

As to the source of the carbon-dioxide, though the lactic acid bacteria which take part in the ripening of cream are sometimes considered to effect a simple change from milk-sugar into lactic acid, according to the equation

$$C_{12}H_{22}O_{11} + H_2O = 4C_3H_6O_3,$$

Fleischmann[1], Richmond[2], and others[3] have pointed out that oxidation to carbon-dioxide takes place as well. I am informed that cream, ripened with the anaerobic lactic acid organism which does not produce gas, requires no ventilation during churning.

The acidity of the cream before and after ripening in each case was estimated by titrating 50 c.c. with decinormal sodium hydrate, using phenolphthalein as indicator, with the following results:

No. of sample	No. of c.c. $\frac{N}{10}$ NaOH required to neutralise 50 c.c. cream			Increase in CO_2 per litre during ripening	Percentage of increased acidity due to CO_2
	Before ripening	After ripening	Increase		
1	7·5	38·2	31·7	181·90 c.c.	12·8 °/。
2	8·0	41·5	33·5	245·55 ,,	16·4 ,,
3	7·0	37·8	30·8	207·45 ,,	15·0 ,,
4	7·9	37·8	29·9	112·95 ,,	8·5 ,,

[1] Fleischmann's *Book of the Dairy*, English translation, pp. 24, 26, 99.

[2] Richmond's *Dairy Chemistry*, pp. 18, 226.

[3] *E.g.* Swithinbank and Newman's *Dairy Bacteriology*, p. 154.

This would indicate that the amount of carbon-dioxide compared with the lactic acid formed is large; but it must be remembered that a very considerable proportion of the latter is neutralised by alkaline phosphates, etc.

Conclusions.

A considerable volume of carbon-dioxide is produced by bacteria during the ripening of cream and held by it in a state of supersaturation.

The agitation in the churn very soon brings about a new condition of equilibrium in accordance with Dalton's law of partial pressures, a large percentage of the carbon-dioxide being liberated. This, together with the slight rise in temperature, is sufficient to account for the necessity to ventilate the churn at intervals during the first few minutes of the process.

The amount of CO_2 produced bears no very constant relation to the lactic acid or to the total acidity.

I have to acknowledge my indebtedness to Mr A. D. Hall, M.A., at whose suggestion and under whose supervision the investigation was carried out, and also to the Lawes Agricultural Trust for the use of the laboratory and apparatus.

THE PRESERVATION OF EGGS BY WATER GLASS AND THE COMPOSITION OF THE PRESERVED EGGS.

By JAMES HENDRICK, B.Sc.,
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One of the most popular and commonly used methods of preserving eggs is by means of water glass. Though this method was introduced only comparatively recently it has largely superseded older methods, and also appears to have led to much more frequent preservation of eggs on the small scale in households and by small traders. The method is simple and effective. The eggs are obtained when they are plentiful and cheap in spring and preserved for use during the winter months. It is therefore necessary to keep them for about six months. In the experiments described below some were left in a solution of water glass as long as four years.

Water glass is a silicate of soda, and is used for a variety of purposes. It is prepared as a thick syrup for use as an egg preservative. In Table I. analyses of two samples are given, and also an analysis of sample 2 diluted with water, for use as an egg preservative. The solutions used in most of my experiments were of about the strength shown in this analysis. The analyses show that silicate of soda used for egg preservation does not contain quite sufficient soda to form the acid metasilicate, $NaHSiO_3$. The solution given by the water glass is strongly alkaline in reaction.

TABLE I.

Composition of Water Glass.

	1.	2.	
		Original	Solution of same used for preserving eggs
Silica	36·87 per cent.	37·91 per cent.	2·76 per cent.
Soda	16·01 ,, ,,	16·48 ,, ,,	1·20 ,, ,,
Potash	0·14 ,, ,,	0·14 ,, ,,	0·01 ,, ,,

A little Carbonate was present in both samples.

Na_2SiO_3 contains Silica 49·18 per cent., Soda 50·82 per cent., Water —,
$NaHSiO_3$,, ,, 60·0 ,, ,, ,, 31·0 ,, ,, ,, 9·0 per cent.

My experiments were at first started only to find out whether eggs would remain for long periods, such as two or three years, in dilute solutions of water glass without undergoing decay or any other serious change of composition. In ordinary practice it is only necessary to preserve the eggs for about six months; my object was to find out whether the eggs would remain good for a much longer period.

Through the kindness of a retail grocer in Aberdeen, who annually preserves a large number of eggs, I was enabled to examine large numbers of eggs which had been preserved in water glass, and also to set aside annually for a number of years small experimental lots of eggs which were kept under the same conditions as those which were preserved for ordinary trade purposes. The eggs were not preserved under the very best conditions, as they were not put into the water glass day by day as they were laid, but were collected in the country and sent into town in large lots before preservation. Most of them therefore were two or three days old before being placed in the preservative. It is therefore the more remarkable that not a single egg in the small experimental lots was ever found to be bad or even tainted. These eggs were of course carefully selected and packed. As was to be expected in the ordinary trade lots, which were preserved in large tubs, a few unsaleable eggs were always found. These were generally chipped or cracked eggs. They had been injured either before being preserved, or during the packing in the preserving tubs. It was very seldom that a really bad, decomposed egg was found, and there is every reason to believe that when any such were found their presence was due to the inclusion of old and tainted eggs among those originally sent in from the country.

The eggs were examined before being put in the preservative, but it is not possible to detect all the slightly decomposed eggs by a mere cursory examination. A note was kept of the total number of unsaleable eggs in certain consignments which were preserved. For instance, in 1905 out of 384 dozen preserved between April and June, and sold between October and December, five dozen, or 1·3 per cent., were bad. The great majority of these were broken or cracked eggs.

Eggs which are preserved in water glass have a nice appearance, as the shells are very clean and fresh looking after the water glass is wiped off them. Even those which had been several years in water glass had a fine fresh appearance. Another advantage of preservation in water glass over certain other methods is that the contents of the egg do not shrink owing to evaporation. The eggs therefore do not rattle when

shaken, no matter how old they are. The cost of preservation is very small.

It was found that eggs which had been kept in water glass for a few months could hardly be distinguished in appearance, flavour and smell, either raw or cooked, from what are called "fresh eggs," that is fresh eggs in the commercial sense, which are eggs which should be free from decomposition or taint, but which may be several days old. A really fresh egg, only a few hours laid, is easily distinguished in flavour and appearance when cooked from the "fresh egg" or preserved egg, and is known as a "new-laid" egg. The eggs which had been preserved in water glass for about six months tasted and smelt like well-kept eggs a few days old. As the eggs in question were a few days old when they went into the water glass, they were not appreciably changed to my eye and palate by a few months' stay in water glass.

As the eggs get older however a distinct change is found which can be appreciated both by the eye and palate. Eggs which have been three or four years in water glass are easily recognised. The white becomes pink in colour and very liquid. The egg acquires a slightly peculiar taste which to my palate suggested soda. At the same time even when four years old the eggs had no unpleasant taste or smell, and the white coagulated in the usual manner in cooking. Though there was a slight characteristic odour when the eggs were cooked, it was not a stale or bad odour and did not suggest sulphuretted hydrogen. The changes in the preserved eggs take place very gradually. At one year old they are hardly noticeable; at two years they are distinct, but not so distinct as at three or four years old.

The above observations, which have no doubt been made by many others, satisfied me that eggs could be preserved in water glass for long periods without decomposing or undergoing any other serious change. As the experiments progressed, however, it was decided to enlarge their scope by determining whether any distinct changes take place in the composition of eggs when they are kept in water glass, and especially whether the soda and silica of the water glass penetrate into the egg to any great extent.

There are comparatively few analyses of eggs, and especially of the ash of eggs, on record. The eggs used all through these experiments were those of the ordinary barndoor fowl. The eggs of ducks, turkeys and other less common fowls were excluded. König (*Menschlichen Nährungs u. Genussmittel*) records a few analyses of eggs, but does not give many ash analyses. Other writers on foods generally quote

König. There are also a few recorded American analyses of eggs, but these do not give ash analyses. I have not succeeded in finding any recorded analyses of preserved eggs, nor am I acquainted with sufficient ash analyses to show what variations may be expected in the different ash constituents of ordinary commercial eggs.

In Tables II. and III. analyses of a number of fresh eggs and of eggs of different ages preserved in water glass are recorded. Owing to an unfortunate accident a portion of the laboratory records on this subject were destroyed, and therefore the analyses of a number of samples are wanting and some of those given in the tables are fragmentary. Each analysis was made on the mixed contents of at least three eggs, and all analyses were made in duplicate.

TABLE II.

Composition of Fresh Eggs.

	Average from König per cent.	1. 3 Eggs per cent.	2. 3 Eggs per cent.	3. 4 Eggs per cent.
Moisture	73·67	73·18	72·70	74·44
Nitrogen	2·01	2·11	—	—
Fat	12·11	10·40	—	—
Ash	1·12	1·02	1·36*	1·06
Ash Soluble in water	—	—	0·55	0·56
Potash	0·159	0·120	0·156	0·139
Soda	0·200	0·194	0·173	(?)
Silica	0·003	0·010	0·021	0·031

* Contained a little carbon.

In Table II. the average analysis of the eggs of barndoor fowls as recorded in König's *Menschlichen Nährungs u. Genussmittel* is given, and then partial analyses made in my laboratory of three samples. It will be seen that the analyses are in general agreement. As the figure for silica is an important one, it was determined carefully in large samples of 40 to 50 gms. of egg.

TABLE III.

Composition of Preserved Eggs.

Preserved / Analysed	1902 / 1904 per cent.	1902 / 1905 per cent.	1903 / 1904 per cent.	1903 / 1905 per cent.	1904 May / 1904 Octr. per cent.
Moisture	72·12	74·66	73·55	73·73	—
Nitrogen	2·17	2·10	2·01	2·07	—
Fat	11·19	9·42	10·70	10·41	—
Ash	0·92	1·00	0·93	1·02	—
Potash	0·075	0·069	0·101	0·073	0·143
Soda	0·296	0·343	0·215	0·311	0·200
Silica	0·023	0·019	0·022	0·039	—

In Table III. partial analyses are given of five samples of preserved eggs. These represent eggs six months, one year, two years, and three years old, and preserved in three different years.

On comparing Tables II. and III. it will be seen that the analyses of the fresh and of the preserved eggs, apart from the details of the ash analyses, are in close agreement. As already pointed out, in water glass the eggs do not dry up, and the tables show that the percentage of moisture in the preserved eggs is quite similar to that in the fresh eggs. There is also practically no change in the percentage of ash. It was anticipated that the ash of the preserved eggs would be greater than that of the fresh eggs, but this anticipation obtains no support from the figures. The ash of the preserved eggs contains no more silica than the ash of the fresh eggs. The oldest sample in Table III. contains less silica than some of the fresh eggs in Table II. We may conclude therefore that silica does not diffuse through the shell into the contents of the egg at all.

The soda slightly increases in the preserved eggs, and the increase appears to be according to the length of time the egg was in water glass. The whole increase in soda however is very slight, and is not comparable with the strength of the soda solution in which the eggs were immersed. Table I. shows that the eggs were immersed in a solution of water glass containing over 1 per cent. of soda. The soda in the eggs which were two or three years in the solution does not appear to have increased more than about 0·1 per cent. There are so few ash analyses of eggs that it cannot be concluded that the apparent increase in soda is not due, in part at least, to natural variations in different samples of eggs. It is remarkable that in Table III. as the soda increases the potash appears to diminish. This may be due to a slow interchange between the potash of the egg and the soda of the solution. But there is another possible explanation, namely, that eggs which are naturally high in soda are low in potash, or that in eggs as in plants, soda and potash are able to replace one another naturally to a certain extent. It is not possible to decide in the absence of extensive series of ash analyses of eggs whether the variation is due to interchange between soda and potash or to natural variations in eggs, but the evidence is so consistent as to make it highly probable that when preserved in water glass the soda of the egg slowly increases, and a very small diminution of the potash takes place. The slight alteration in the flavour of the egg and in the liquidness of the white may be due to the increase in soda.

The alkalinity of the contents of the eggs appeared to increase with the length of time they were in water glass, but the increase was small, and in a complicated substance like egg it was found difficult to measure it accurately.

The general conclusion to be drawn from Tables II. and III. is that there is practically no change in the composition of eggs even from lengthened immersion in water glass. Practically no silica and very little, if any, soda find their way into the eggs.

Analyses of the shells of a number of samples of eggs were also made chiefly in order to determine whether much silica was deposited in the shell. The results of these analyses are given in Table IV. In all cases the shells were those of eggs the contents of which had been removed for analysis (Tables II. and III.). The shells were not washed out in any way and therefore always had a little of the white adhering to them. They were allowed to stand in air in a dry, warm place till quite air-dry and brittle, but were not dried in an oven. The shells, including the membranes and any white sticking to them, were then ground and mixed. Each sample consisted of the shells of at least three eggs.

TABLE IV.

Composition of Shells.

Fresh Eggs in Preservative	From / To	Spring 1905 / Spring 1906	Spring 1903 / Summer 1905	Spring 1902 / Summer 1905
Moisture	3·45	2·95	3·12	2·85
Combustible Matter	5·13	4·32	8·60	8·47
Ash	91·42	92·72	88·28	88·68
Lime	49·05	49·16	48·15	48·15
Equal to Carbonate of Lime	87·59	87·78	85·98	83·98
Silica	0·57	1·64	1·95	2·32
Percentage of Lime in Ash	53·65	53·02	54·54	54·30

The considerable differences in organic matter in the different samples are probably largely due to different quantities of white adhering to different samples. If we omit the different quantities of organic matter adhering to the shells, the main difference between the different samples is in the amount of silica which they contain. In the fresh eggs this is about ½ per cent., and it increases according to the length of time the eggs have been in the solution. In the eggs which were three years in the solution the silica amounts to nearly 2½ per cent. It appears then that a slow deposition of silica takes place in the shell of the egg. The percentage of lime in the shells remains practically constant.

This deposition of silica in the shells probably blocks up the pores of the shells to some extent and renders them less permeable.

MICROBIOLOGIE AGRICOLE, pp. 439. E. KAYSER.

(J. B. BAILLIÈRE, Paris, 1905.)

DURING the last quarter of a century much light has been thrown by mycologists upon the relation of micro-organisms to the fertility of the soil. No concise and yet adequate account of their researches has hitherto been offered to the student of agriculture either in this country or on the continent. Conn's *Agricultural Bacteriology* fails to cover the ground, and the information in the translations of Alfred Fischer's *Vorlesungen über Bakterien*, and in the first edition of Lafar's *Handbuch der Technischen Mykologie* is scattered and insufficient.

M. Kayser, Microbiologist to the National Institute for Agriculture of France, devotes half of his book to an account of the mycology of the soil, and if one includes the chapters on 'ensilage' and 'milk' two-thirds of the work is of direct interest to the student of agriculture.

The book is divided into three parts: (1) an introduction dealing with bacteria in general; (2) the relation of micro-organisms to the fertility of the soil; and (3) microbiology applied to the transformation of (*a*) vegetable and (*b*) animal products.

The second part discusses the distribution of micro-organisms in the cultivated soil, their rôle in the fermentation of farm-yard manure, nitrification, denitrification, nitrogen fixation, the purification of sewage and industrial waste waters and finally the 'sulphur' and 'iron' bacteria. The whole of this account is excellent, and does not neglect the results of the most recent work. Dealing with farm-yard manure the author clearly distinguishes the aerobic from the anaerobic changes, and gives special notice to the specific organisms bringing about the fermentations of cellulose and urea. He assumes, however, both in this connexion and again when dealing with 'the septic tank' that methane is only produced from cellulose or similar bodies, whereas it was shown long ago that the gas is also formed during the putrefaction of proteids.

In the section on 'nitrification' a detailed account is given of the

method employed by Winogradsky for isolating the nitrous and nitric organisms and of their behaviour in artificial culture. The recent work of Boullanger and Massol, which has gone a good way towards clearing up the symbiotic relations of the two organisms under natural conditions, is also fully discussed. On page 99 the figures quoted from Wolff showing the amounts of nitric nitrogen in various soils to a depth of 20 centimetres are surely exceedingly high. A reference is made to the thermo-chemistry of nitrification, and the two following equations given:

$$2NH_3 + 6O = 3H_2O + N_2O_3 + 157 \text{ cal.}$$

$$N_2O_3 + 2O = N_2O_5 + 37 \text{ cal.}$$

One would like to know which 'calorie' is here employed. The author does not attempt to calculate out the amount of energy available from nitrous oxidation for decomposing carbonic acid, although Winogradsky's figures, showing that about 36 parts of ammoniacal nitrogen are oxidised for every part of carbon fixed, are quoted. It is stated that '*nitrosomonas*' forms a surface film on solutions in pure culture, but there is no reference to this property in Winogradsky's recent account of nitrification for the second edition of Lafar's *Handbuch*. We are also informed that the oxidation of ammonia to nitrous acid is due to an 'oxidase'; this may well be so, but it has not yet been proved.

Dealing with 'nitrogen fixation' three conditions are distinguished, viz. fixation by free-living soil organisms—*Clostridium Pasteurianum* and *Azotobacter*, fixation by symbiosis of algae and bacteria, and by symbiosis of bacteria with leguminous and certain other green plants. In connexion with fixation of nitrogen by Beijerinck's '*Azotobacter*' the author says that the Dutch mycologist considers many species of bacteria to fix free nitrogen and divides them into oligo-, macro- (*sic*) and polynitrophiles according to their activity for fixation. A reference to Beijerinck's paper shows this as a misquotation, his classification being into oligo- micro-, and polynitrophiles according to their ability to grow on media containing little or much combined nitrogen. There is a good account of 'nitrogen fixation' by leguminous plants, although some of Hiltner's recent speculative but stimulating notions are not clearly separated from his facts.

In the third part a description is given of the most important moulds, yeasts, and bacteria concerned in the industries closely allied to agriculture, and then a short account of the microbiology of the industries themselves, viz. brewing, distilling, vinification, cider-making, production of vinegar, starch manufacture, baking, retting of flax,

preparation of tobacco, tanning, and especially the preparation of ensilage and milk products.

In the introduction the author has something to say about enzymes, and again in the chapter on ensilage, but he calls them by the confusing name of 'diastases' after the custom of his fellow-countrymen.

There is a classification of bacteria, very simple, antiquated and inaccurate, in which 'bacillus' is distinguished from 'bacterium' as a longer and narrower rod; one notes also a contradiction, for after characterising the genus 'Sarcina' as 'endospore bearing' and including it in Coccaceae, the statement is made two pages later that the Coccaceae never have spores.

The book is very well illustrated from microphotographs and drawings, although the magnification is in many cases not given. The index might have been more extensive and there is no bibliography, but that was hardly to be expected in a work of the size.

If only for the sake of the part on 'the microbiology of the soil' this little book can be strongly recommended to the agricultural student who reads French, and a translation would be a useful addition to the English text-books of agricultural science.

S. F. ASHBY.